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A novel ratiometric probe based on rhodamine B and coumarin for selective recognition of Fe(III) in aqueous solution

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ABSTRACT

We have developed a new ratiometric fluorescence probe based on rhodamine B and coumarin to monitor the Fe^{3+} with high sensitivity and selectivity. Upon addition of Fe^{3+} to aqueous solution of the probe, two fluorescence peaks at 580 nm and 460 nm were observed, which belong to rhodamine B and coumarin, respectively. This is a novelty design of ratiometric probe of Fe^{3+}, due to CHEF process generated along with the PET process suppressed simultaneously. The fluorescence intensity at 580 nm was significantly increased about 120-fold with 5 equiv. of Fe^{3+} added in aqueous solution.

1. Introduction

Fe^{3+} plays a major role in many biochemical processes at the cellular level. High levels of Fe^{3+} within the body have been associated with increasing incidence of certain cancers and dysfunction of certain organs, such as the heart, pancreas, and liver [1,2]. Accordingly, the development of methods, enabling professionals to spatially and temporally track intracellular Fe^{3+}, is challenging but essential to address these issues and has become the subject of current chemical research.

Optical cellular imaging with fluorescent probes might be the best choice for visualizing the intracellular Fe^{3+} ion by virtue of its high sensitivity, high-speed spatial analysis, and less cell-damaging. However, most reported examples of fluorescent sensing of Fe^{3+} ions in living cells were functioned through the enhancement of fluorescence signals [3–5]. As the change in fluorescence intensity is the only detection signal, factors such as instrumental efficiency, environmental conditions, and the probe concentration can interfere with the signal output [6,7]. Ratiometric probes can eliminate most or all interferences by built-in correction of two emission bands and seem to be more favorable for imaging intracellular metal ions in comparison with fluorescence enhancement probes.

Ratiometric probes can be designed to function following two mechanisms: intramolecular charge transfer (ICT) [8–11] and fluorescence resonance energy transfer (FRET) [12–16]. Relatively broad fluorescence spectra are often observed for ICT fluorophores; in a significant number of cases the broad fluorescence spectra before and after binding target ions have a high degree of overlap (or in an extreme case, a broad spectrum with high intensity completely covers one with lower intensity, which makes it difficult to accurately determine the ratio of the two fluorescence peaks. However, probes based on FRET can avoid these disadvantages in a certain extent [7,17]. Although some fluorescent probes for detecting Fe^{3+} have been reported [18–21], up to now, only a few ratiometric probe for Fe^{3+} was reported [19,22] based on cyclodextrin supramolecular complex and quinoline, respectively. Moreover, the synthesis process of the probe based on cyclodextrin superamolecular is complex, and the fluorescence intensity of quinoline acted as donor is week.

Thus, we designed a coumarin-rhodamine system **L** as a ratiometric probe for Fe^{3+}.

Two fluorescence peaks which belong to coumarin and rhodamine exist simultaneously. Upon addition of Fe^{3+}, a new fluorescence emission peak appeared at 580 nm. This wavelength change allows the ratiometric detection of Fe^{3+} ions in ethanol/water solution. To the best of our knowledge, this is a novelty design of ratiometric probe of Fe^{3+} based on the conjugated link of the rhodamine and coumarin. Herein, we develop a new

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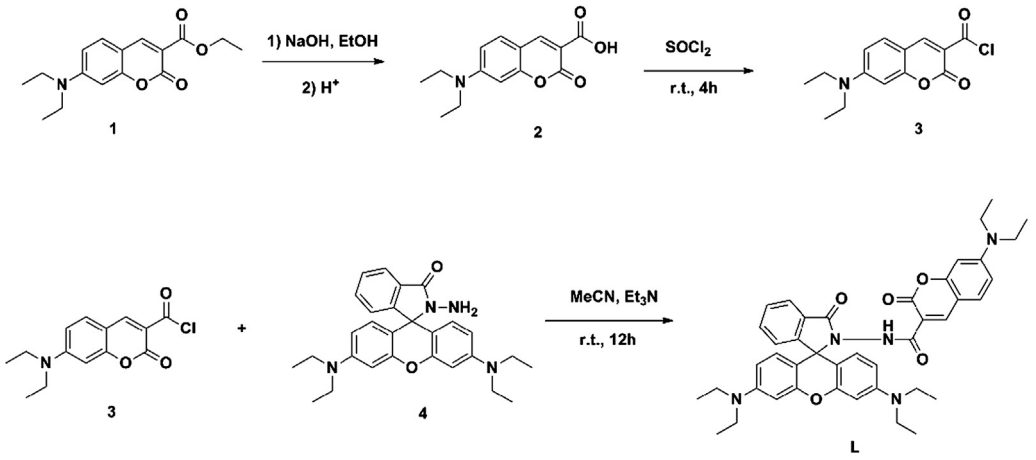


Scheme 1. Synthesis of probe **L**.

ratiometric probe **L** for Fe^{3+}, which differs from reported ratiometric probe.

2. Experimental sections

2.1. Materials and methods

Deionized water was used throughout the experiment. All the reagents were purchased from commercial suppliers and used without further purification. All samples were prepared at room temperature, shaken for 10 s and waited for 18 h before UV–vis and fluorescence determination. The solutions of metal ions were prepared from $NaNO_3$, $Mg(NO_3)_2{\cdot}6H_2O$, KNO_3, $Ca(NO_3)_2{\cdot}4H_2O$, $Cr(NO_3)_3{\cdot}9H_2O$, $Fe(NO_3)_3{\cdot}9H_2O$, $Co(NO_3)_2{\cdot}6H_2O$, $Ni(NO_3)_2{\cdot}6H_2O$, $Cu(NO_3)_2{\cdot}3H_2O$, $Zn(NO_3)_2{\cdot}6H_2O$, $AgNO_3$, $Cd(NO_3)_2{\cdot}4H_2O$, $Ba(NO_3)_2$, $HgCl_2{\cdot}H_2O$, $Pb(NO_3)_2$, respectively, and were dissolved in distilled water. Thin-layer chromatography (TLC) was conducted on silica gel $60F_{254}$ plates (Merck KGaA). HEPES buffer solutions (pH 7.2) were prepared using 20 mM HEPES, and proper amount of aqueous sodium hydroxide under adjustment by a pH meter. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 300 spectrometer, using DMSO as solvent and tetramethylsilane (TMS) as internal standard. Melting points were determined on an XD-4 digital micro melting point apparatus. IR spectra were recorded with an IR spectrophotometer VERTEX 70 FT-IR (Bruker Optics). HRMS spectra were recorded on a Q-TOF6510 spectrograph (Agilent). UV–vis spectra were recorded on a U-4100 (Hitachi). Fluorescent measurements were recorded on a PerkinElmer LS-55 luminescence spectrophotometer.

2.2. Synthesis of ethyl 7-(diethylamino)-2-oxo-2H-chromene-3-carboxylic acid (2)

A mixture of **1** (1.15 g, 4 mmol) and NaOH (0.48 g 12 mmol) in EtOH (20 mL) was refluxed for 1 h, after which an orange solid was precipitated. When the reaction was cooled to room temperature, the mixture was added to a breaker containing 150 mL water. The orange solid was dissolved and adjust the pH of the solution to 3 ~ 4. An orange solid 7-(diethylamino) coumarin-3-carboxylic acid **2** (0.845 g) was obtained in 81% yield; m.p. 90–92 °C.

2.3. Synthesis of 7-(diethylamino)-2-oxo-2H-chromene-3-carbonyl chloride (3)

The acid chloride **3** was synthesized by the reaction of the 7-(diethylamino) coumarin-3-carboxylic acid **2** with thionyl chloride at room temperature in 80% yield.

2.4. Synthesis of N-(3′,6′-bis(diethylamino)-3-oxo-4a',9a'-dihydrospiro [isoindoline-1,9′-xanthen]-2-yl)-7-(diethylamino)-2-oxo-2H-chromene-3-carboxamide (L)

Compound **3** (0.279 g, 1.0 mmol) and **4** (0.547 g, 1.2 mmol) was dissolved in 20 mL of acetonitrile. The mixture was stirred for 8 h under nitrogen at room temperature. Concentration of the mixture under reduced pressure gave a yellow solid. The residue was purified by silica gel column using petroleum ether: ethyl acetate = 1:1 as eluent to obtain an orange solid **L** (0.51 g) in 73% yield; m.p. 305 °C. IR (KBr, cm^{-1}): 3461.7, 2971.4, 2928.4, 1725.4, 1692.3, 1615.9, 1582.3, 1512.7, 1353.4, 1215.8, 1117.1, 819.0, 787.6; ^1H NMR (300 MHz, DMSO-d_6) δ (ppm) 1.05–1.15 (18H, m, CH_3), 3.30–3.38 (8H, m CH_2), 3.44–3.51 (4H, m, CH_2), 6.33 (2H, d, J = 2.4 Hz, ArH), 6.37–6.40 (2H, m, ArH), 6.57 (1H, d, J = 1.8 Hz, ArH), 6.62 (1H, s, ArH), 6.65 (2H, s, ArH), 6.78–6.82 (1H, m, ArH), 7.02–7.05 (1H, m, ArH),7.51–7.56 (2H, m, ArH), 7.57-7.65 (1H, m, ArH), 7.84–7.87 (1H, m, NH), ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm) 12.4, 43.6, 65.2, 97.2, 103.8, 107.0, 118.0, 128.5, 132.0, 145.0, 148.4, 149.0, 152.8, 157.5, 161.1, 162.2, 164.0; HRESIMS calcd for $[M + H]^+$ $C_{42}H_{46}N_5O_5^+$: 700.3499 found: 700.3422.

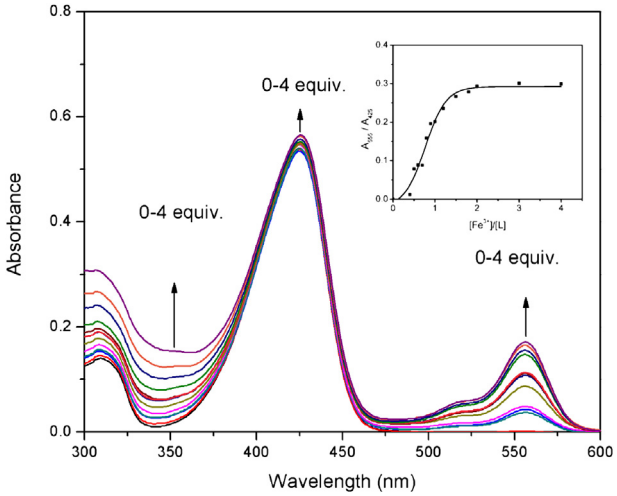


Fig. 1. Absorption spectra of 10 μM **L** upon the addition of Fe^{3+} (0–4 equiv.) in buffered EtOH/HEPES = 99:1 solution at pH 7.2. The inset shows the ratio of the absorbance (555 nm/425 nm) as a function of Fe^{3+} concentration.

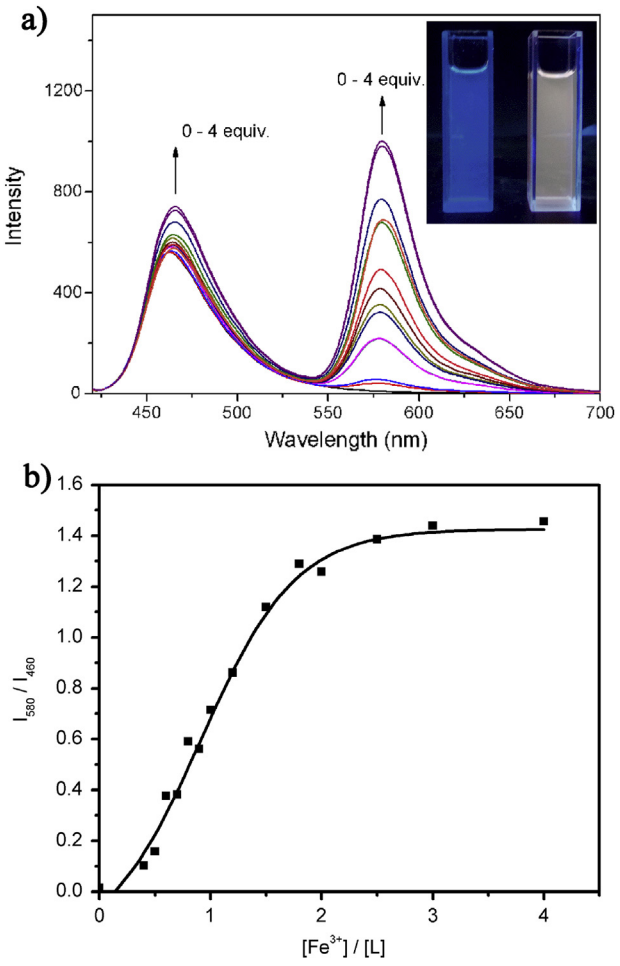


Fig. 2. a) The fluorescence spectra of 10 μM **L** upon the addition of 0–4 equiv Fe^{3+} in buffered EtOH/HEPES = 99:1 solution at pH 7.2. The inset shows the fluorescence photo of **L** and **L**-Fe^{3+}. b) The ratio of fluorescence intensity (580 nm/460 nm) of **L** as a function of Fe^{3+} concentration.

3. Result and discussion

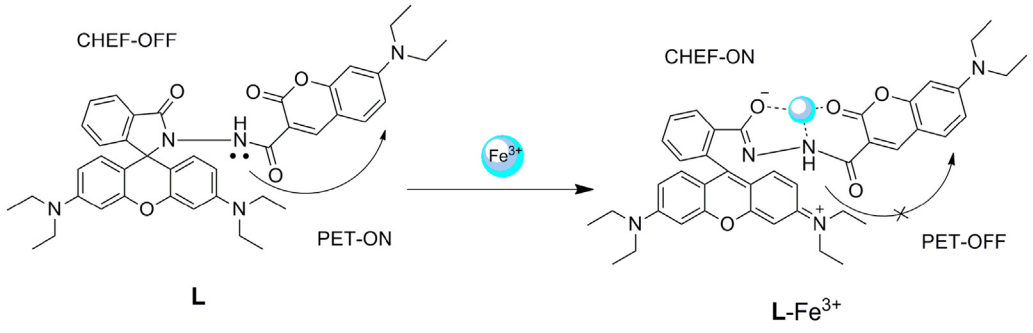
Probe **L** was synthesized by the reaction of 7-(diethylamino)-2-oxo-2H-chromene-3-carbonyl chloride **3** and rhodamine B hydrazine **4** in ethanol at room temperature for 12 h in 73% yield (Scheme 1). Compound **3** was easily obtained by the reaction of 7-(diethylamino)-2-oxo-2H-chromene-3-carboxylic acid **2** and sulfurous dichloride at room temperature for 4 h. Compound **4** was synthesized according to our previous report [23]. The structure of **L** was determined by IR, ^{1}H NMR, ^{13}C NMR and HRMS. The spirolactam form of the rhodamine B units was confirmed by the presence of a peak at ~65.2 ppm in the ^{13}C NMR spectra.

The UV/Vis spectrum of **L** solution (EtOH/HEPES 99:1, pH 7.2) showed only the absorption profile of the coumarin at 425 nm, which indicates that **L** exists as a spirocycle-closed form (Fig. S1) [24,25]. An obvious absorption at 560 nm induced by addition of Fe^{3+} confirmed the ring-opened of rhodamine. We can also find that upon addition of Cu^{2+}, Co^{2+}, Ni^{2+} and Fe^{3+}, the solution of **L** display an obvious jacinth color at the micromolar level and other ions show little interference (Fig. S2).

To get more insight on the binding mode of **L** and Fe^{3+}, absorption spectra titration was performed as shown in Fig. 1. It can be observed that the absorbance peaks at 425 nm and 555 nm increased upon gradual addition of Fe^{3+} and remained constant after 2 equiv. of Fe^{3+} added. A significant color change from green to pink could be observed easily by naked-eyes (Fig. S2). The nonlinear fit of the data revealed that the binding of **L** to Fe^{3+} was most probably of 1:1 stoichiometry with an association constant (Ka) of about Ka = 1.172×10^4 M^{-1} (Fig. S3). This binding mode was also supported by the data of Job's plots evaluated from the absorption spectra of **L** and Fe^{3+} with a total concentration of 20 μM (Fig. S4).

Excitation at 425 nm introduced an emission at 460 nm. Upon addition of Fe^{3+}, a new fluorescence peak appeared at 580 nm attributed to rhodamine B part (Fig. 2). With the addition of Fe^{3+} the peaks at 460 nm and 580 nm increased gradually. And the ratio of fluorescence intensity at 580 nm and 460 nm (I_{580nm}/I_{460nm}) peaked when 2 equiv. Fe^{3+} was added. The fluorescence clearly changed from blue to orange. Finally, the concurrence of fluorescence of rhodamine B part (λ_{em} = 580 nm, red color) and coumarin part (λ_{em} = 460 nm, blue color) resulted in an orange color. Moreover, a 155 nm of strokes shift (425 nm–580 nm) of probe **L** can eliminate the interference of most or all ambiguities by self-calibration of two emission bands. The solution of **L**-Fe^{3+} possesses a quantum yield of 0.33 in EtOH/H_2O (1:1, v/v, pH 7.2) and 0.38 in ethanol, based on optically matching solution of rhodamine B standard (ΦF = 0.69 in ethanol) at an excitation wavelength of 425 nm and emission at 580 nm. The detection limit, based on the definition by IUPAC (*CDL* = 3*Sb*/*m*), is 5.24×10^{-7} M^{-1}, which can be obtained from the liner of response range covering a concentration range of Fe^{3+} from 4.0×10^{-6} to 10.0×10^{-6} M^{-1} (Fig. S5). The fluorescence intensity enhanced approximately 120 fold at 580 nm.

According to the theory of FRET, coumarin excimer emission should decrease when such energy transfer takes place. However, unexpected phenomenon was observed (Fig. 2). To explain the phenomenon, a mechanism is proposed (Scheme 2). For coumarin part, the *N* atom with an unshared electron pair quenches both the coumarin monomer and excimer emissions strongly in the absence of Fe^{3+} due to a photoinduced electron/energy transfer (PET) process [26,27]. However, comparing with literature, the fluorescence



Scheme 2. Proposed complex mechanism of **L**-Fe^{3+}.

was not quenched completely, so the PET process was not obvious. When Fe^{3+} is added to the solution of **L**, the *N* atom participates in the coordination process of probe **L** and Fe^{3+}. So PET process is suppressed and the fluorescence increased. For rhodamine part, fluorescence also increased, due to the opening of spirolactam and chelation-enhanced fluorescence (CHEF). Thus, for the fluorescence phenomenon of the **L**-Fe^{3+} complex, CHEF may play a more important role than FRET [28]. Enhanced fluorescence of both the coumarin monomer and rhodamine B upon addition of Fe^{3+} is observed. So the fluorescence of rhodamine (~580 nm) and coumarin (~460 nm) enhanced simultaneously.

Probe **L** shows an excellent selectivity toward Fe^{3+}. The fluorescence spectra (λ_{ex} = 425 nm) of **L** with respective metal cations are shown in Fig. 3a. Without cation, **L** revealed no fluorescence at 500–650 nm. In fact, **L** also did not give any observable response for many metal ions such as Mg^{2+}, Na^{+}, Mn^{2+}, Ni^{2+}, Pb^{2+}, Zn^{2+}, Ba^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, or K^{+}. However, addition of Fe^{3+} created a remarkable fluorescence enhancement at 580 nm and a little enhancement at 460 nm. A ratio of fluorescence intensity at 580 nm vs. 460 nm was shown in Fig. 3b. Fe^{3+} can be detected clearly by probe **L**. Moreover, an ability to resist interference is an important index for a cationic probe. The enhancement of the fluorescence intensity resulting from the addition of Fe^{3+} was little influenced by subsequent addition of other metal ions except Cu^{2+} and Ni^{2+} (Fig. S6). However, the solution of **L**-Fe^{3+} turned a deeper red upon addition of Cu^{2+} and Ni^{2+}, which can be distinguished well by naked eyes. All of these results indicated the high selectivity of **L** for Fe^{3+} over other competing cations in EtOH/H_2O (99:1, v/v, pH 7.2) solution.

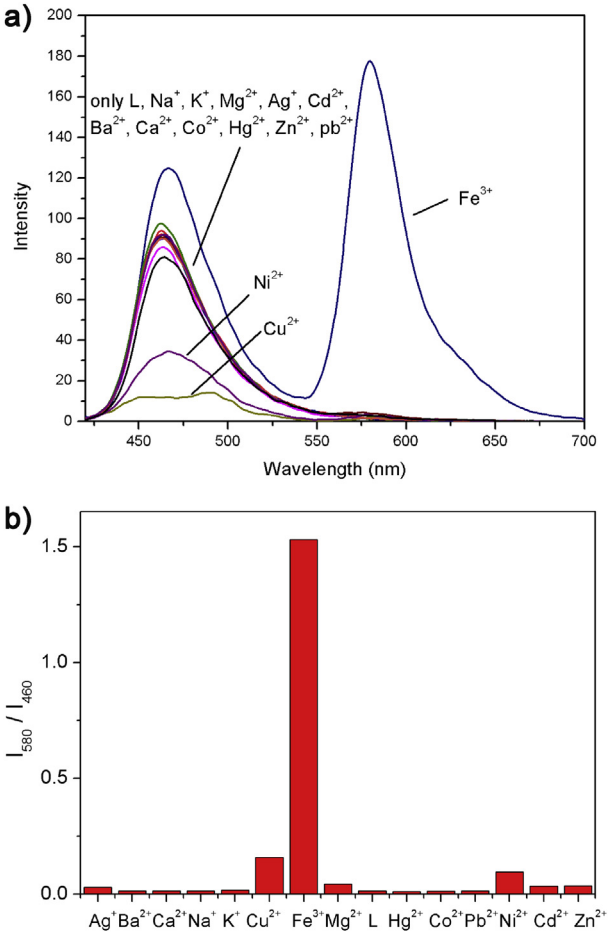


Fig. 3. a) The fluorescence spectra of 10 μM **L** in buffered EtOH/HEPES = 99:1 solution at pH 7.2 with 5 equiv. metal ions: Ba^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, K^{+}, Mg^{2+}, Na^{+}, Ag^{+}, Fe^{3+}, Pb^{2+}, Hg^{2+}, Ni^{2+}, Cu^{2+} ions and blank. b) The ratio of fluorescence intensity (580 nm/460 nm) of **L** and **L** with metal ions.

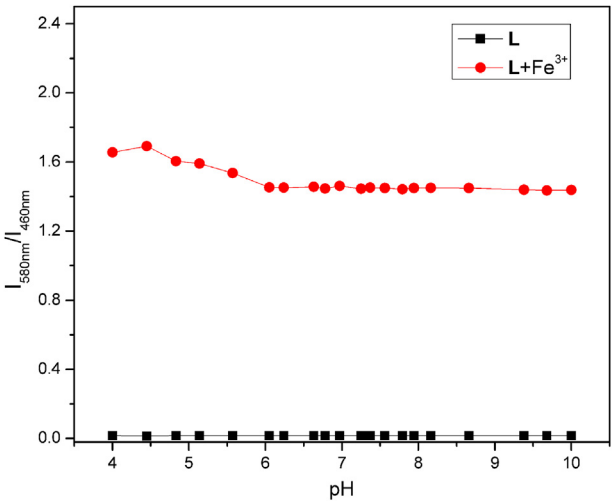


Fig. 4. The effect of pH (4.0–10.0) on the fluorescence intensity ratio (580 nm/460 nm) of 10 μM probe **L** with 5.0 equiv. Fe^{3+} in buffered EtOH/HEPES solution.

A pH titration showed that probe **L** had stable fluorescence property over a wide pH span of 4–10 (Fig. 4), which suggests that probe **L** is suitable for application under physiological conditions. The curves (Fig. S7) of time response showed that the probe can complex with 5 equiv. Fe^{3+} completely in 7 min, after which the fluorescence intensity peaks.

4. Conclusion

In summary, we developed a novel ratiometric fluorescence probe based on rhodamine and coumarin. The probe displayed a high sensitivity and selectivity toward Fe^{3+} even coexistent with other metal ions. The probe differs from reported ratiometric probe in fluorescence mechanism.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.dyepig.2013.06.024.

References

[1] D'Autreáux B, Tucker NP, Dixon R, Spiro S. A non-haem iron centre in the transcription factor NorR senses nitric oxide. Nature 2005;437:769–72.
[2] Lee JW, Helmann JD. The PerR transcription factor senses H_2O_2 by metal catalysed histidine oxidation. Nature 2006;440:363–7.
[3] Xiang Y, Tong A. A new rhodamine-based chemosensor exhibiting selective Fe^{III}-amplified fluorescence. Org Lett 2006;8:1549–52.
[4] Weerasinghe AJ, Schmiesing C, Varaganti S, Ramakrishna G, Sinn E. Single- and multiphoton turn-on fluorescent Fe^{3+} sensors based on bis(rhodamine). J Phys Chem B 2010;114:9413–9.
[5] She M, Yan Z, Yin B, Zhang J, Gu J, Yin W, et al. A novel rhodamine-based fluorescent and colorimetric "off-on" chemosensor and investigation of the recognizing behavior towards Fe^{3+}. Dye Pigme 2012;92:1337–43.
[6] Zhang X, Xiao Y, Qian X. A ratiometric fluorescent probe based on FRET for imaging Hg^{2+} ions in living cells. Angew Chem Int Ed 2008;47:8025–9.
[7] Han ZX, Zhang XB, Li Z, Gong YJ, Wu XY, Jin Z. Efficient fluorescence resonance energy transfer-based ratiometric fluorescent cellular imaging probe for Zn^{2+} using a rhodamine spirolactam as a trigger. Anal Chem 2010;82:3108–13.

[8] Xu Z, Qian X, Cui J. Colorimetric and ratiometric fluorescent chemosensor with a large red-shift in emission: Cu(II)-only sensing by deprotonation of secondary amines as receptor conjugated to naphthalimide fluorophore. Org Lett 2005;7:3029–32.
[9] Yang H, Liu Z, Zhou Z, Shi E, Li F. Highly selective ratiometric fluorescent sensor for Cu(II) with two urea groups. Tetrahedron Lett 2006;47:2911–4.
[10] Liu Z, Zhang C, He W, Yang Z, Gao X, Guo Z. A highly sensitive ratiometric fluorescent probe for Cd^{2+} detection in aqueous solution and living cells. Chem Commun 2010;46:6138–40.
[11] Goswami S, Sen D, Das NK. A new highly selective, ratiometric and colorimetric fluorescence sensor for Cu^{2+} with a remarkable red shift in absorption and emission spectra based on internal charge transfer. Org Lett 2010;12:856–9.
[12] Kim HJ, Park SY, Yoon S, Kim JS. FRET-derived ratiometric fluorescence sensor for Cu^{2+}. Tetrahedron 2008;64:1294–300.
[13] Lee SY, Kim HJ, Wu JS, No K, Kim JS. Metal ion-induced FRET modulation in a bifluorophore system. Tetrahedron Lett 2008;49:6141–4.
[14] Zhou Z, Yu M, Yang H, Huang K, Li F, Yi T. FRET-based sensor for imaging chromium(III) in living cells. Chem Commun 2008;29:3387–9.
[15] Long L, Lin W, Chen B, Gao W, Yuan L. Construction of a FRET-based ratiometric fluorescent thiol probe. Chem Commun 2011;47:893–5.
[16] Sun Y, Zhong C, Gong R, Mu H, Fu E. A ratiometric fluorescent chemodosimeter with selective recognition for sulfite in aqueous solution. J Org Chem 2009;74:7943–6.
[17] Liu Y, Lv X, Zhao Y, Chen M, Liu J, Guo W. A naphthalimide-rhodamine ratiometric fluorescent probe for Hg^{2+} based on fluorescence resonance energy transfer. Dye Pigme 2012;92:909–15.
[18] Mao J, He Q, Liu W. A rhodamine-based fluorescence probe for iron(III) ion determination in aqueous solution. Talanta 2010;80:2093–8.
[19] Wang S, Meng X, Zhu M. A naked-eye rhodamine-based fluorescent probe for Fe(III) and its application in living cells. Tetrahedron Lett 2011;52:2840–3.
[20] Dalapati S, Jana S, Alam MA, Guchhait N. Multifunctional fluorescent probe selective for Cu(II) and Fe(III) with dual-mode of binding approach. Sensor Actuat B 2011;160:1106–11.
[21] Liu SR, Wu SP. New water-soluble highly selective fluorescent chemosensor for Fe (III) ions and its application to living cell imaging. Sensor Actuat B 2012;171–172:1110–6.
[22] Xu M, Wu S, Zeng F, Yu C. Cyclodextrin supramolecular complex as a water-soluble ratiometric sensor for ferric ion sensing. Langmuir 2010;26:4529–34.
[23] Liu WY, Li HY, Zhao BX, Miao JY. Synthesis, crystal structure and living cell imaging of a Cu^{2+}-specific molecular probe. Org Biomol Chem 2011;9:4802–5.
[24] Xiang Y, Tong A, Jin P, Ju Y. New fluorescent rhodamine hydrazone chemosensor for Cu(II) with high selectivity and sensitivity. Org Lett 2006;8:2863–6.
[25] Li M, Lu HY, Liu RL, Chen JD, Chen CF. Turn-on fluorescent sensor for selective detection of Zn^{2+}, Cd^{2+}, and Hg^{2+} in water. J Org Chem 2012;77:3670–3.
[26] Li Z, Zhang L, Li X, Guo Y, Ni Z, Chen J, et al. A fluorescent color/intensity changed chemosensor for Fe^{3+} by photo-induced electron transfer (PET) inhibition of fluoranthene derivative. Dye Pigme 2012;94:60–5.
[27] Sung K, Fu HK, Hong SH. A Fe^{3+}/Hg^{2+}-selective anthracene-based fluorescent PET sensor with tridentate ionophore of amide/β-amino alcohol. J Fluoresc 2007;17:387–9.
[28] Othman AB, Lee JW, Wu JS, Kim J, Abidi S, Thuéry RP, et al. Calix[4]arene-bsed, Hg^{2+}-iduced intramolecular fluorescence resonance energy transfer chemosensor. J Org Chem 2007;72:7634–40.