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A highly sensitive, selective ratiometric fluorescent probe for cobalt (II) and its applications for biological imaging†

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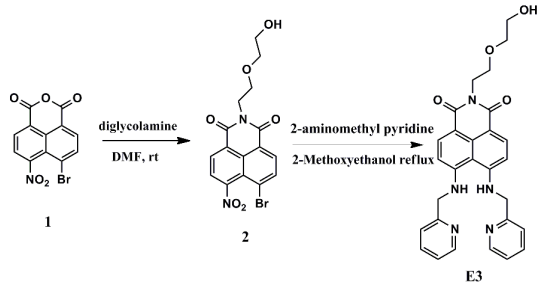
Probe E3 has been developed as the first ratiometric fluorescent cobalt probe with high sensibility and selectivity based on internal charge transfer (ICT). Most importantly, the probe achieved the imaging and detection of cobalt in cells with ratiometric measurement.

As is well known, cobalt is a critical trace element existed in cobalamin and other metalloproteins, such as B_{12} vitamin with a presence of cobalt in 4.35%.[1] The total contents of cobalt in human body is 1.1 - 1.5 mg , of which 43% stored in muscle tissue, 14% in the bone, and the other 43% in other soft tissue.[2] Additionally, cobalt is also indispensable in some biological process such as hematopoiesis, the metabolism of fats, the formation of a few proteins, amino acids, erythrocytes, coenzyme and myelin, and the performance of glandula thyreoidea.[3] But, exposure to excessive amount of cobalt can cause a series of healthy issues, such as mutagenesis, cardiotoxicity, asthma, lung fibrosis, lung cancer and even inhibit some enzyme activities.[4] Therefore, more attention should be paid on the development of method to detect trace amounts of cobalt samples both in the environment and *in vivo*. Owing to the high selectivity, sensitivity and operational simplicity, fluorescent probes have attracted much attention in detecting ions and neutral molecules in *vivo* and *vitro* in recent years. [5] In particular, ratiometric measurements have the important advantage that they permit signal rationing, and thus could increase the dynamic range and provide a built-in correction for environmental effects. [6] Because of the fluorescence quenching nature of paramagnetic Co(II), non-quenched fluorescent probes for Co(II) are very scarce,[7] and there were a few literatures reported for the ratiometric fluorescent detction of Co(II).[8] However, to the best of our knowledge, none of them could be used for the detection of Co(II) *in vivo*. Herein, we reported a highly sensitive, selective and simple-synthetic ratiometric fluorescent cobalt probe **E3** based on internal charge transfer (ICT) mechanism.



Scheme 1 The synthetic procedure of probe **E3**

The synthesis of probe **E3** is shown in Scheme 1. Reaction of 4-bromo-5-nitro-1, 8-anhydride naphthalene **1** with diglycolamine in DMF afforded **2**, and then further reacted with 2-aminomethyl pyridine in ethylene glycol monomethyl ether to give **E3** in 40 % yield. The structure of **E3** was confirmed by ^{1}H NMR, ^{13}C NMR and HR-MS spectra (See SI†).

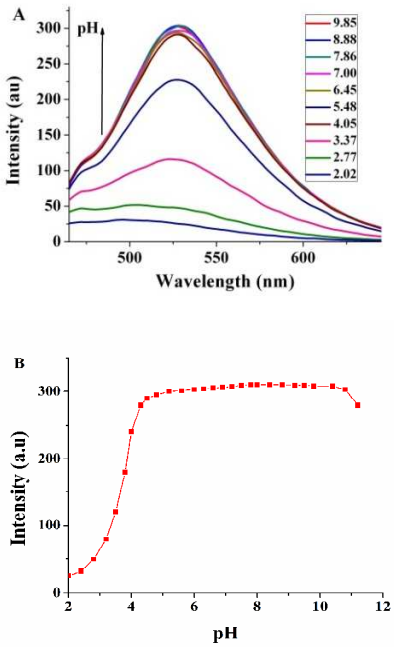


Fig. 1 A) Fluorescence pH titration spectra of **E3** in H_2O/EtOH (v/v 60/40). B) Influence of pH on the fluorescence at 528 nm. λ_{ex} = 440 nm.

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As shown in Fig. S1† and S2†, **E3** has the maximum absorption peak at 457 nm, and its corresponding maximum emission peak was at 528 nm. The new emission peak at 474 nm was due to the decreased internal charge transfer (ICT) effect after the binding of **E3** to the target metal ions [9]. All photochemical experiments were carried out with the excitation wavelength at 440 nm. Furthermore, we evaluated the influence of pH value on the fluorescence emission of **E3** (Fig. 1), and the results showed that there was no influence on the fluorescence emission with pH ranging from 5.0 to 10.0. Thus, all photochemical experiments were carried out in 50 mM HEPES/EtOH (v/v: 60/40) buffer at pH 7.2.

Fig. 2 showed the changes in the fluorescence emission spectra of **E3** as a function of Co^{2+} concentrations. It was noted that, with the increase of the Co^{2+} concentration to 50 × 10^{-6} M, the emission intensity at 528 nm gradually decreased from 272 to 75, and at the same time, a new emission peak at 474 nm appeared, which afterwards increased from 82 to 235, and that was a nearly 10-fold increasement at the ratio of I_{474}/I_{528}. Moreover, the fluorescence ratio of I_{528}/I_{474} changed in a linear fashion with the concentration of Co^{2+} from 1.0 to 10.0 × 10^{-6} M (linearly dependent coefficient: R^2 = 0.978, Figure 2B). It indicated that probe **E3** can be potentially used to quantitatively detect the Co^{2+} concentration. According to the literature report [10] and Figure 2B, the detecting limit of Co^{2+} is 1.0 × 10^{-6} M at 1.0 × 10^{-5} M of **E3** in 50 mM HEPES/EtOH (v/v : 60/40) buffer at pH 7.2. A Job's plot indicated that **E3** chelated Co^{2+} ion with 1:1 stoichiometry (Fig. S4†). The association constant Ks was determined as 1.1 × 10^7 (Fig. S5†).

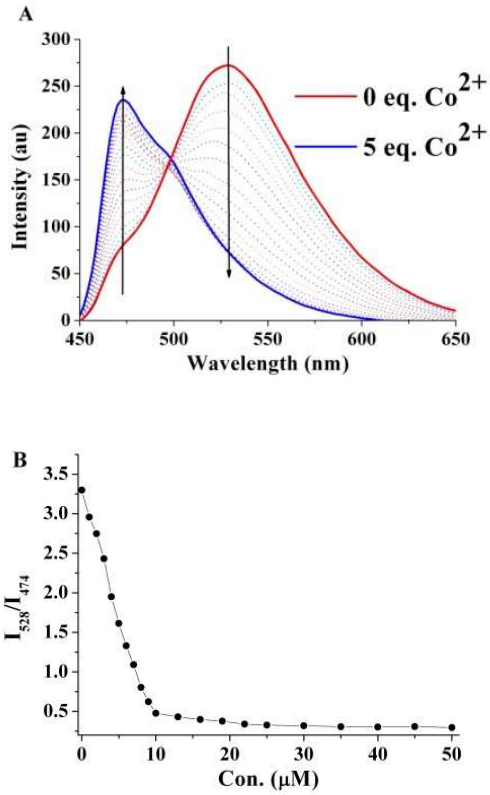

Fig. 2 (A) The changes in the fluorescence spectra of **E3** (10 × 10^{-6} M in 50 mM HEPES/EtOH (v/v : 60/40) buffer, pH 7.2) upon titration with $Co(ClO_4)_2$ from 1.0 × 10^{-6} M to 50 × 10^{-6} M. (B) The fluorescence intensity of **E3** versus the Co^{2+} concentration at I_{528}/I_{474} nm.

The coordination of **E3** to Co^{2+} was also explored by minimize energy molecular modeling. The Co^{2+} in the complex is coordinated by the four nitrogen atoms of the two aminomethyl pyridine arms in which the amino group coordinated to the Co^{2+} and caused the ICT process. To certify the complex modeling, we ran the ESI-MS of the resulting solution by addition of Co^{2+} into **E3** solution and found a peak from ESI-MS with a m/z of 555.1299 (Fig. S6†), while the molecular weight of $[\mathbf{E3}+Co^{2+}-H^+]^+$ was calculated to be 555.1306, thus it was demonstrated that **E3** chelated Co^{2+} ion with 1:1 stoichiometry.

To evaluate the selectivity of the fluorescent probe **E3** toward Co^{2+}, competition experiments were also performed. As shown in Fig. 3A, nearly no fluorescence increase was observed at 474 nm with Li^+, K^+, Zn^{2+}, Ce^{2+}, Fe^{2+}, Fe^{3+}, Hg^{2+}, Mg^{2+}, Cd^{2+}, Pb^{2+}, Mn^{2+}, Ca^{2+}, Na^+, Ni^{2+} and Cu^{2+}. In contrast, Co^{2+} significantly enhanced the fluorescence intensity at 474 nm and as a result, the ratio of **E3** at I_{474}/I_{528} was the highest. Furthermore, when 3.0 equiv of other metal ions was separately added to **E3** solution in the presence of 1.0 equiv Co^{2+}, the fluorescence ratio I_{474}/I_{528} did not change obviously compared with that of Co^{2+} alone (Fig. 3B).

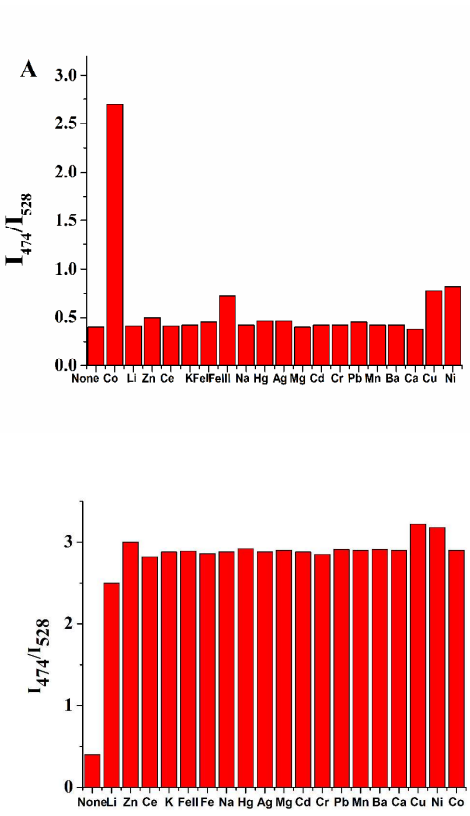

Fig. 3 (A) The fluorescence ratio of **E3** (10 × 10^{-6} M) at I_{474}/I_{528} in the presence of different metal ions (3.0 equiv.). (B) the fluorescence ratio of **E3** at I_{474}/I_{528} with 3.0 equiv. metal ions (Li^+, Zn^{2+}, Ce^{2+}, K^+, Fe^{2+}, Fe^{3+}, Na^+, Hg^{2+}, Ag^+, Mg^{2+}, Cd^{2+}, Cr^{3+}, Pb^{2+}, Mn^{2+}, Ba^{2+}, Ca^{2+}, Ni^{2+} Cu^{2+}), followed by 1 equiv. Co^{2+}. All experiments were performed in 50 mM HEPES/EtOH (v/v : 60/40) buffer at pH 7.2.

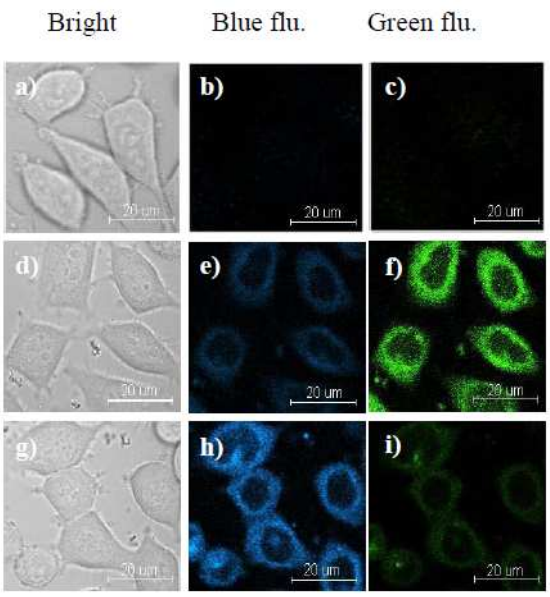

Fig. 4 Confocal fluorescence images of living HeLa cells using a 440 nm laser exciting. (a), (b), (c): HeLa cells incubated with none probe and Co^{2+}. (d), (e), (f): HeLa cells incubated with 10×10^{-6} M **E3**. (g), (h), (i): HeLa cells incubated with 10×10^{-6} M **E3**, then 5 equiv. Co^{2+} was added after 30 mins incubation and washed with PBS contain 2 % EtOH. The bule fluorescence collected the emission wavelength at the range of 470 to 480 nm, and the green fluorescence collected the emission wavelength at the range of 525 to 535 nm. All cells were rinsed three times with 0.1 M PBS buffer contain 2 % EtOH at room temperature before imaging

Subsequently, to further demonstrate the practical application potential of the probe **E3** in cell imaging. HeLa cell lines were chosen for these studies due to its wide applications in cell studies. As shown in Fig. 4, the normal HeLa cells showed no fluorescence. Upon loading of the probe **E3** into the HeLa cell medium, weak blue fluorescence and strong green emission were observed. After incubated with extra Co^{2+} (50 μM) for several minutes, an enhanced intense blue fluorescence and a decreased green fluorescence were surveyed, which reveal the whole ICT process as shown in Fig. 2A. Therefore, **E3** could be used as a practical fluorescent probe for the measurement of Co^{2+} both *in vitro* and in living cells. Meanwhile, to the best of our knowledge, it was the first flourescent probe reported for the detection of Co^{2+} in living cells.

Conclusions

In summary, a novel fluorescent probe **E3** was designed and synthesized and its optical properties as well as its application potentials were evaluated. It specifically responded to Co^{2+} with high selectivity and sensitivity under physicological neutral conditions. Notably, probe **E3** was demonstrated to be efficient and practical for the detection of Co^{2+} in living cells. Improtantly, it was the first flourescent probe that could achieve the detection of Co^{2+} in living cells.

Acknowledgements

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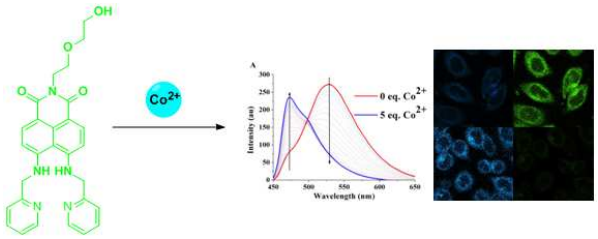

A novel fluorescent probe **E3** was designed and synthesized and evaluated. It responded to Co^{2+} with high selectivity and sensitivity under physicological neutral conditions specifically. Furthermore, notably, probe **E3** was demonstrated for detection of Co^{2+} in living cells, indicative of its practical application potential.