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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 sensitivity 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 B12 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 thyroidea.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 detection 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 1H NMR, 13C NMR and HR-MS spectra (See SI†).

Fig. 1 A) Fluorescence pH titration spectra of E3 in H2O/EtOH (v/v 60/40). B) Influence of pH on the fluorescence at 528 nm. λex = 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 decrease in 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 Co²⁺ concentrations. It was noted that, with the increase of the Co²⁺ concentration to 5.0 × 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 103-fold increase of the Co²⁺ concentration to 50 × 10⁻⁴ M (linearly dependent coefficient: R² = 0.978, Figure 2B). It indicated that probe E3 could potentially be used to quantitatively detect the Co²⁺ concentration. According to the literature report 8 and Figure 2B, the detecting limit of Co²⁺ is 1.0 × 10⁻⁶ M at 1.0 × 10⁻⁴ 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 Co²⁺ ion with 1:1 stoichiometry (Fig. S4†). The association constant Kₛ was determined as 1.1 × 10⁻⁷ M⁻¹ (Fig. S5†).

The coordination of E3 to Co²⁺ was also explored by minimize energy molecular modeling. The Co²⁺ in the complex is coordinated by the four nitrogen atoms of the two aminomethyl pyridine arms in which the amino group coordinated to the Co²⁺ and caused the ICT process. To certify the complex modeling, we ran the ESI-MS of the resulting solution by addition of Co²⁺ into E3 solution and found a peak from ESI-MS with a m/z of 555.1299 (Fig. S6†), while the molecular weight of [E3+Co²⁺-H⁺]⁺ was calculated to be 555.1306, thus it was demonstrated that E3 chelated Co²⁺ ion with 1:1 stoichiometry.

To evaluate the selectivity of the fluorescent probe E3 toward Co²⁺, competition experiments were also performed. As shown in Fig. 3A, nearly no fluorescence increase was observed at 474 nm with Li⁺, K⁺, Zn²⁺, Ce³⁺, Fe³⁺, Fe⁴⁺, Hg²⁺, Mg²⁺, Cd²⁺, Pb²⁺, Mn²⁺, Cu²⁺, Na⁺, Ni²⁺ and Cu²⁺. In contrast, Co²⁺ significantly enhanced the fluorescence intensity at 474 nm and as a result, the ratio of E3 at I₄₅₇/I₅₂₈ 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 Co²⁺, the fluorescence ratio I₄₅₇/I₅₂₈ did not change obviously compared with that of Co²⁺ alone (Fig. 3B).

![Fig. 2](image_url)

**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(ClO₄)₂ from 1.0 × 10⁻⁴ M to 50 × 10⁻⁴ M. (B) The fluorescence intensity of E3 versus the Co²⁺ concentration at I₄₅₇/I₅₂₈ nm.

![Fig. 3](image_url)

**Fig. 3** (A) The fluorescence ratio of E3 (10 × 10⁻⁶ M) at I₄₅₇/I₅₂₈ in the presence of different metal ions (3.0 equiv.). (B) the fluorescence ratio of E3 at I₄₅₇/I₅₂₈ with 3.0 equiv. metal ions (Li⁺, Zn²⁺, Ce³⁺, K⁺, Fe³⁺, Fe⁴⁺, Na⁺, Hg²⁺, Ag⁺, Mg²⁺, Cd²⁺, Cr³⁺, Pb²⁺, Mn²⁺, Ba²⁺, Cu²⁺, Ni²⁺, Cu²⁺), followed by 1 equiv. Co²⁺. All experiments were performed in 50 mM HEPES/EtOH (v/v: 60/40) buffer at pH 7.2.
Potentials were evaluated. It specifically responded to Co(II), the detection of Co(II) was synthesized and its optical properties as well as its application for the detection of Co(II) were surveyed, which reveal the whole ICT process as shown in Fig. 2A. Intense blue fluorescence and a decreased green fluorescence were observed for a fluorescent probe that could achieve detection of Co(II).

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(II) with high selectivity and sensitivity under physiological neutral conditions. Notably, probe E3 was demonstrated to be efficient and practical for the detection of Co(II) in living cells. Importantly, it was the first fluorescent probe that could achieve the detection of Co(II) in living cells.

Conclusions

In summary, a novel fluorescent probe E3 was designed and its optical properties as well as its application potentials were evaluated. It specifically responded to Co(II) with high selectivity and sensitivity under physiological neutral conditions. Notably, probe E3 was demonstrated to be efficient and practical for the detection of Co(II) in living cells. Importantly, it was the first fluorescent probe that could achieve the detection of Co(II) in living cells.

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Notes and references

2. R. Olinescu and M. Greabu, Mecanisme de aparare a organismului impotriv poluari chimice, Ed. Tehnica, Bucuresti, 1990 (Romania).
A novel fluorescent probe E3 was designed and synthesized and evaluated. It responded to Co\(^{2+}\) with high selectivity and sensitivity under physiological neutral conditions specifically. Furthermore, notably, probe E3 was demonstrated for detection of Co\(^{2+}\) in living cells, indicative of its practical application potential.