Electronic Supplementary Information

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

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1. Materials and general methods:

N, N′–dimethylformamide (DMF) was distilled from calcium hydride (CaH₂) under anhydrous condition. Other solvents were of analytic grade. All reactions were carried out under a helium atmosphere with analytic grade solvents, unless noted. Mass spectra were measured on a HP 1100 LC-MS spectrometer. Double distilled water was used to prepare all aqueous solutions. All spectroscopic measurements were performed in 50 mM HEPES/EtOH (v/v: 60/40) buffer at pH 7.2. Fluorescence spectra were determined on a VARIAN CARY Eclipse Fluorescence spectrophotometer. Absorption spectra were determined on a VARIAN CARY 100 Bio UV-Visible spectrophotometer. 1H NMR and 13C NMR were measured on a BrukerAV-400 spectrometer with chemical shifts reported in ppm (in CDCl₃; TMS as internal standard). All pH measurements were made with a Sartorius basic pH-Meter PB-10. All reactions were monitored by thin-layer chromatography (TLC) using UV-light (254 nm) and Flu-light (365 nm). Silica gel (300 - 400 mesh) was used for column chromatography.

2. Synthesis:

![Scheme 1 Synthesis of fluorescent probe E3.](image)

**Preparation of 1:**
Compounds 1 was prepared according to the reported procedure.¹

**Preparation of 2:**
To a solution of compound 1 (500 mg, 1.55 mmol) in EtOH, diglycolamin (163 mg, 1.55 mmol) was added, and the mixture was stirred refluxing for 2 h. Then the mixture cooled to room temperature, and concentrated in vacuo to afford yellow oil crude compounds. The crude product was purified by column chromatography (silica gel: 100 mL, eluent: DCM/MeOH 50/1) to afford yellow powder 2 in 60 % yield. 1H NMR (400 MHz, CDCl₃, 20 °C): δ 8.73 (d, J = 8.0 Hz, 1 H), 8.53 (d, J = 8.0 Hz, 1 H), 8.23 (d, J = 8.0 Hz, 1 H), 7.94 (d, J = 8.0 Hz, 1 H), 4.45 (t, J = 5.6 Hz, 5.2 Hz, 2 H), 3.87 (t, J = 5.2 Hz, 5.6 Hz, 2 H), 3.70 – 3.66 (m, 4 H), 2.1 (s, 1H).

**Preparation of E3:**
To a solution of compound 2 (205 mg, 0.50 mmol) in ethylene glycol monomethyl ether, 2-Aminomethyl pyridine (542 mg, 5.01 mmol) was added, and the mixture was stirred at reflux for 6 hours.

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h. Then the mixture cooled to room temperature, and concentrated in vacuo to afford yellow oil crude compounds. The crude product was purified by column chromatography (silica gel: 100 mL, eluent: DCM/MeOH 20/1) to afford yellow powder E3 in 42 % yield. \(^1\)H NMR (400 MHz, CDCl\(_3\), 20°C): \(\delta\) 8.44 (d, \(J = 8.4\) Hz, 2 H), 8.31 (d, \(J = 4.8\) Hz, 2 H), 7.73 – 7.67 (m, 4 H), 7.41 (d, \(J = 7.6\) Hz, 2 H), 7.21 – 7.18 (m, 2 H), 6.80 (d, \(J = 8.8\) Hz, 2 H), 4.68 (s, 2 H), 4.66 (s 2 H), 4.42 (t, \(J = 5.6\) Hz, 5.6 Hz, 2 H), 3.86 (t, \(J = 5.6\) Hz, 5.6 Hz, 2H), 3.76 (s, 1 H), 3.72 – 3.68 (m, 4 H). \(^{13}\)C NMR (100 MHz, CDCl\(_3\), 20°C): \(\delta\) 170.74, 169.34, 164.96, 156.01, 152.06, 149.04, 136.84, 134.06, 122.52, 121.86, 111.71, 107.08, 72.23, 68.84, 61.91, 49.15, 39.08. HRMS (ESI\(^+\)): m/z calcd for: C\(_{28}\)H\(_{28}\)N\(_5\)O\(_4\)\(^+\): 498.2141, found: [M+H]\(^+\) 498.2147.

3. Supplementary data:

Figure S1. The absorption spectra of E3 with different concentrations in 50 mM HEPES/EtOH (v/v : 60/40) buffer at pH 7.2.

Figure S2. The fluorescence emission spectra of E3 with different concentrations in 50 mM HEPES/EtOH (v/v : 60/40) buffer at pH 7.2.
**Figure S3.** The changes in the absorption 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 × 10⁻⁶ M to 50 × 10⁻⁶ M.

**Figure S4.** Job plot analysis of E3 and Co²⁺ in 50 mM HEPES/EtOH (v/v : 60/40) buffer at pH 7.2; The total molar concentration of E3 and Co²⁺ is 1.0×10⁻⁵ M.

**Figure S5.** The fitting curve of fluorescence intensity at I₄₇₄ nm/I₅₂₈ nm of E3 versus increasing concentrations of Co²⁺ in water/EtOH solution (v/v : 60/40, 50 mM HEPES buffer, pH 7.2). The concentration of E3 was 10×10⁻⁶ M.
Figure S6. The ESI-MS assay on detection of Co$^{2+}$ with probe E3

Figure S7. $^1$H NMR spectrum of E3
Figure S8. $^{13}$C NMR spectrum of E3

Figure S9. HRMS spectrum of E3