A Red-shift Colorimetric and Fluorescent Sensor for Cu$^{2+}$ in Aqueous Solution: Unsymmetrical 4,5-Diaminonaphalimide with N-H Deprotonation Induced by Metal Ion

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S1. Reagents and general methods

1.1 Reagents

All the reagents and solvents were commercial available and used without purification. ¹H-NMR spectra were collected in CDCl₃ at 25 °C on a Bruker AV-400 spectrometer. Electrospray ionization (ESI) spectroscopy was performed in Mass Instrumentation Facility of Analysis & Research Center of ECUST.

UV-vis spectra were obtained using a Varian Cary 100 spectrophotometer (1 cm quartz cell) at room temperature; Fluorescence spectra were obtained using a Varian Cary Eclipse fluorescent spectrophotometer (1 cm quartz cell) at room temperature; Melting points were determined by using an Büchi Melting point B-540 apparatus (uncorrected when using); pH was measured with a Sartorius pH-Meter PB-20.

The metal salts employed are LiClO₄, NaClO₄, KClO₄, Mg(ClO₄)₂, Cd(ClO₄)₂·6H₂O, Hg(ClO₄)₂·3H₂O, Mg(ClO₄)₂, Cr(ClO₄)₃·6H₂O, FeCl₃, FeSO₄·7H₂O, Zn(ClO₄)₂·6H₂O, AgClO₄·H₂O, Co(ClO₄)₂·6H₂O, Mn(ClO₄)₂·6H₂O, Cu(ClO₄)₂·6H₂O, Ni(ClO₄)₂·6H₂O, and Pb(ClO₄)₂·3H₂O, respectively.

Metal ions and sensors were dissolved in water or ethanol to obtain 1 mM stock solutions.

1.2 Association Constants and Curve fitting

The following equation was used for the nonlinear least squares analysis to determine the binding constant (Kₐ):

\[
Y = Y₀ + \frac{Y_{\text{lim}} - Y₀}{2} \left\{ 1 + \frac{C_M}{C_L} + \frac{1}{Kₐ C_L} - \left[ \left( 1 + \frac{C_M}{C_L} + \frac{1}{Kₐ C_L} \right)^2 - 4 \frac{C_M}{C_L} \right]^{1/2} \right\}
\]

Here, \( Y \) was the recorded fluorescent intensity, \( Y₀ \) was the start value without the addition of Cu²⁺ ion, \( Y_{\text{lim}} \) was the limiting value (left as a floating parameter), \( C_M \) was the Cu²⁺ concentration, and \( C_L \) was the sensor concentration. (B. Valeur, *Molecular Fluorescence: Principle and Applications*, Wiley-VCH, Germany, 2002)
S2 Spectra ($^1$HNMR, $^{13}$CNMR, HRMS) of H1

6-(bis(2-hydroxyethyl)amino)-2-butyl-7-(pyridin-2-ylmethylamino)-1H-benzo[d]isooquinoline-1,3(2H)-dione
S3. The ESI(+)-Ms of H1 and Cu²⁺ (equimolar H1/Cu2+, 2 μM/2 μM) in water/ethanol (9/1) solution.

Elemental Composition Report

Multiple Mass Analysis: 6 mass(es) processed
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 50.0
Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron ions
40 formula(e) evaluated with 4 results within limits (up to 50 closest results for each mass)

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S4. Influence of pH on the absorption spectra of H1/Cu²⁺ adduct

Fig. S4. (a). Influence of pH on the absorption spectra of H1/Cu²⁺ adduct (5 μM H1 and 5 μM Cu²⁺) in ethanol-water solutions (1/9, v/v). (b) Influence of pH from 1.77 to 4.23. (c) Influence of pH from 4.23 to 6.75. d) Influence of pH from 6.75 to 12.45.

S5. Influence of pH on the fluorescent spectra of H1/Cu²⁺ adduct

Fig. S5. (a) Influence of pH on fluorescent spectra of H1/Cu²⁺ adducts (5 μM H1 and 5 μM Cu²⁺) in ethanol-water solutions (1/9, v/v). (b) Influence of pH from 1.77 to 4.23. (c) Influence of pH from 4.23 to 6.75. (d) Influence of pH from 6.75 to 12.45.
S6. Cu$^{2+}$-titration at pH 11.59 in aqueous ethanol (ethanol/water = 1/9) solution

**Fig. S6.** Cu$^{2+}$ ion titration-induced the fluorescent spectra changes of sensor H1 (10 μM) at pH11.59 in aqueous ethanol (ethanol/water = 1/9) solution (Cu$^{2+}$ amount was 0.25, 0.5, 0.75, 1.0 equiv respectively).