A red-shift colorimetric and fluorescent sensor for Cu2+ in aqueous solution: Unsymmetrical 4,5-diaminonaphthalimide with N-H deprotonation induced by metal ions

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A red-shift colorimetric and fluorescent sensor for Cu^{2+} in aqueous solution: unsymmetrical 4,5-diaminonaphthalimide with N-H deprotonation induced by metal ions†

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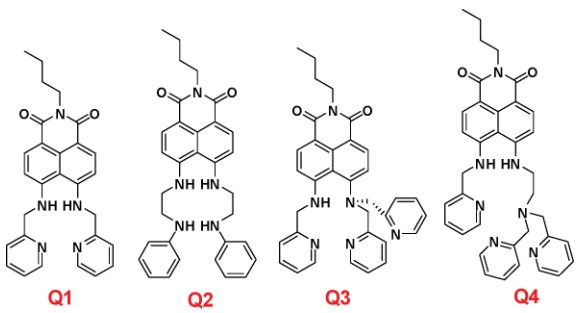
4,5-Diamino-1,8-naphthalimide (DNP)-based chemosensor **H1** was designed and synthesized. Probe **H1** specifically recognized Cu^{2+} ions in neutral aqueous solution. The capture of Cu^{2+} by the receptor resulted in deprotonation of the secondary amine conjugated to the 1,8-naphthalimide chromophore, so that the electron-donation ability of the "N" atom would be greatly enhanced; thus probe **H1** showed a 50 nm red-shift in absorption (from 464 nm to 514 nm) and a large colorimetric response, it also exhibited an on-off fluorescent response. Specifically, **H1** is a pH-independent sensor in the range of 6.0 to 12.0 and can be used as a probe for Cu^{2+} in strong basic conditions. These results opened up new possibilities for construction of red-shift colorimetric and fluorescent sensors.

Introduction

Recently, there has been considerable interest in molecule and ion recognition based on supramolecular chemistry.[1] Fluorescent and colorimetric chemosensors for various analytes have been developed for application in physiology and medical diagnostics.[1,2] Such devices can give rise to real time, noninvasive, and on-line monitoring *in vitro* or *in vivo*.[1a–d,3] Thus, we are interested in this field and use 4-monoamino-1,8-naphthalimide (MNP) and 4,5-diamino-1,8-naphthalimide (DNP) as chromophores to develop chemosensors.[4]

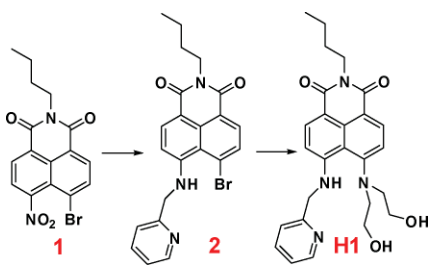
1,8-Naphthalimide with an electron donor and an acceptor (EDA) group is characteristic of an internal charge transfer (ICT) chromophore.[4–6] In this system, the energetics of the ICT state can be modulated by changing the strength of the donor and acceptor groups. An increase in strength of the donor group is expected to result in an increase of charge separation and an enhancement of dipole moment, and then to decrease the energy of the ICT state. Spectroscopically, this is manifested in a red shift of the electronic absorption spectrum and fluorescent emission.[1a,4–6]

4,5-Diamino-naphthalimide (DNP) and its derivatives are versatile candidates for constructing a fluoroionophore system; some chemosensors based on this moiety have been originally developed by our group[4] as showed in Scheme 1. Compared with the parent compound MNP, the 4,5-disubstituted DNP system possesses: (1) a relatively rigid and nice cation binding pocket composed of two nitrogen fragments in the naphthalimide moiety and its extended sidechains;[5d] (2) intrinsic cation-induced deprotonation of the N-H fragment, which strengthened the donor-π-acceptor push-pull character and shifted the absorption and emission to long wavelengths, that is, gave rise to large colorimetric or ratiometric fluorescence signal responses;[4c–e,6,7] (3) the tunable selectivity through changing the type and amount of substituted-amine in an asymmetry or symmetry arrangement.



Scheme 1 Sensors derived from 4,5-diamino-1,8-naphthalimide (DNP) were developed by Qian's group.[4b–d]

With these considerations, an asymmetrical multidentate sensor **H1** (Scheme 2) was designed by incorporating 2-aminomethylpyridine and 2-diethanolamine fragments to DNP as the recognition receptor. One secondary amine unit remains to perform the deprotonation process which makes the sensor work as a colorimetric and fluorescent probe.[4c–e] Poly-sidechains or multidentate ligands,[4d,e,g,8] here 2-aminomethylpyridine and 2-diethanolamine, allow the sensor to selectively recognize targets. The two-hydroxyl units ensure better water compatibility of the senor, which is very important for the application of **H1** in biological



Scheme 2 Synthesis of **H1**.

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† Electronic supplementary information (ESI) available: Spectra (^{1}H-NMR, ^{13}C-NMR, ESI-MS) and UV/vis pH-titration curve. See DOI: 10.1039/b818611a

systems and environment analysis.[5f] In addition, the OH group, which is harder than the pyridine N, shows a weaker tendency to coordinate with metal ions than the pyridine N (according to the soft and hard theory).[9] This further affords **H1** high selectivity in sensing metal ions.

Results and discussion

Synthesis

Sensor **H1** was synthesized as shown in Scheme 2. Firstly, by reacting **1** (4-bromo-5-nitro-1,8-naphthalimide)[10] with 2-aminomethylpyridine, the monosubstitution product **2**[4b] was given, and then with secondary amine (2-diethanolamine), the target compound **H1** was obtained (with 21% yield).‡

pH-titration and spectral responses

First the pH response of **H1** in ethanol-water solution (1/9, v/v) was evaluated. When the pH value was in the range of 12.0–4.5, the absorption spectra of **H1** (Fig. 1) remained unaffected. When the pH varied from 4.5 to 2.0, the absorption intensity gradually decreased (~9%), accompanied by a 13 nm blue-shift (464 nm to 451 nm). This was mainly due to the electronic effect between the donor group "NH" and the positively charged pyridine moiety upon protonation, that is, the protonated pyridine decreased the electron donation ability along the methylene of the 2-picolyl moiety and then weakened the ICT (intramolecular charge transfer) process, which led to the blue shift in absorption.[1f,4b,d] The fluorescence of free **H1** at 543 nm (λ_{ex} = 464 nm) also showed no change in the pH range of 12.0–4.5, then it decreased (~40%) with a 12 nm blue shift (from 543 nm to 531 nm) when the pH changed from 4.5 to 2.0. The pK_a was 4.0 derived from the pH-titration curves (Fig. 2b).[4d,e] These changes in fluorescence were similar to the analogues (**Q1** and **Q3** in Scheme 1) due to the combination of the photoinduced electron transfer (PET) process from the naphthalimide fluorophore to the protonated pyridine group and the weakened ICT process as shown in Scheme 3.[1f,4b,d,e] Therefore further investigation was carried out in HEPES (20 mM, pH 7.05) buffer solution (containing 10% ethanol as cosolvent).

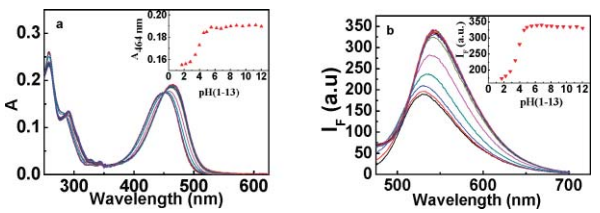


Fig. 1 Influences of pH on the absorption (a) and fluorescence (b) of **H1** (10 μM) in the ethanol-water solution (1/9, V/V). The pH is modulated by adding 75% $HClO_4$ or NaOH solution.

‡ The synthesis of **H1**: 6-(bis(2-hydroxyethyl)amino)-2-butyl-7-(pyridin-2-ylmethylamino)-1H-benzo[de]isoquinoline-1,3(2H)-dione. Under N_2 gas conditions, **2** (preparation according to the literature[4e]) (140 mg, 0.32 mmol), diethanolamine (525 mg, 5.0 mmol) were combined in 5 mL of 2-methoxyethanol and refluxed for 8 h, then poured into 50 mL water and extracted with DCM (50 mL×3). The combined DCM was dried over anhydrous Na_2SO_4 and was then evaporated to afford a yellow oil. The oil was flushed through a silica column (500:25 = DCM/methanol) and dried to yield the yellow solid product (22 mg, 14.9%), m.p. 211.3 °C. ^{1}H-NMR ($CDCl_3$, 400 MHz), δ 0.88 (3H, t, J = 7.2 Hz), 1.32–1.37 (2H, m), 1.57–1.64 (2H, m), 3.26–3.41 (4H, m), 3.66–3.68 (2H, m), 3.78–3.81 (2H, m), 4.06 (2H, t, J = 7.2 Hz), 4.63 (2H, s), 5.65 (2H, br), 6.51 (2H, d, J = 8.8 Hz), 7.20–7.24 (1H, m), 7.50 (2H, d, J = 8.0 Hz), 7.78 (1H, t, J = 7.6 Hz), 8.26 (1H, d, J = 8.4 Hz), 8.45 (1H, d, J = 3.2 Hz), 8.49 (1H, d, J = 8.0 Hz), 11.90 (1H, br). ^{13}C-NMR ($CDCl_3$, 400 MHz), δ 13.87, 20.41, 30.24, 39.88, 47.44, 58.76, 59.27, 103.91, 109.10, 117.69, 120.59, 122.30, 123.21, 123.90, 131.91, 132.19, 134.74, 138.28, 148.87, 152.58, 157.14, 157.61, 164.21, 164.31. HRMS (ESI) Calcd for MH^+, 463.2345; Found, 463.2301.

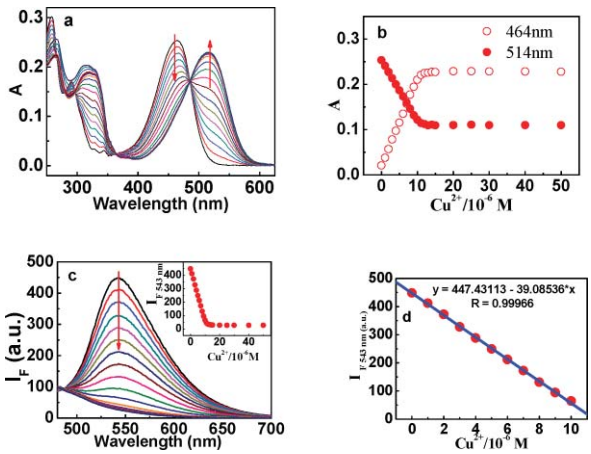
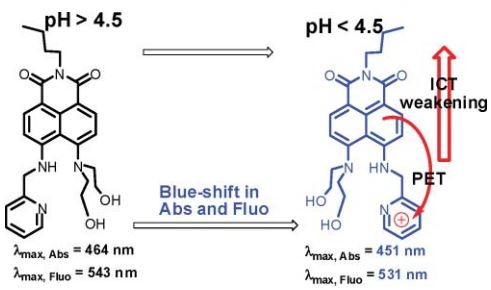


Fig. 2 (a) Cu^{2+}-titration induced the absorption spectra change of sensor **H1** (10 μM) in HEPES (20 mM, pH 7.05) buffer (containing 10% ethanol as the cosolvent). (b) Absorption intensity of **H1** at 464 nm (solid) and 514 nm (open) as a function of Cu^{2+} concentration. (c) Cu^{2+}-titration induced the fluorescence spectra (λ_{ex} = 464 nm) change of sensor **H1** (10 μM) in HEPES (20 mM, pH 7.05) buffer (containing 10% ethanol as the cosolvent). Inset: fluorescent intensity of **H1** at 543 nm (solid) as a function of Cu^{2+} ion concentration (λ_{ex} = 464 nm, $\lambda_{,em}$ = 543 nm). (d) The linear dependence of **H1** within the concentration of Cu^{2+} (0.0–10.0 μM).



Scheme 3 Representative scheme explaining changes in absorption and fluorescence.

Cu^{2+}-titration and spectral responses

The responses of **H1** to Cu^{2+} were investigated in detail as shown in Fig. 2. The absorption spectra of free **H1** exhibited a maximum centered at 464 nm which indicated that the pyridine was unprotonated in HEPES (20 mM, pH 7.05) buffer solution as demonstrated above. Upon sequential addition of Cu^{2+} to the sensor solution, the band at 514 nm (2.30×10^4 $M^{-1}cm^{-1}$) increased in intensity at the expense of the 464 nm (2.54×10^4 $M^{-1}cm^{-1}$) transition as shown in Fig. 2a. Plotting the changes in absorption intensity at 464 nm (●) and 514 nm (○) as a function of Cu^{2+} ion indicated a 1:1 stoichiometry between **H1** and Cu^{2+} (Fig. 2b).

There was a large 50 nm red shift in absorption from 464 nm to 514 nm with a clear isosbestic point at 485 nm and a color change from yellow to pink which was clearly evident to the naked eye. Considering that the DNP-based sensor had the potential feature of metal ion-induced deprotonation of the NH fragment,[4b,d,e] it could be reasoned that the red-shift in absorption was originated from the Cu^{2+}-induced deprotonation of the "NH" moiety, that is, the deprotonated "N^-" enhanced the ICT character and resulted in the 50 nm red shift in absorption. Similar observations were reported previously in its analogues[4d,e] and in some 1,8-naphthalimide based-anion sensors.[5d–g,6,7] It was further confirmed by HRESI(+)-MS experiments.[11] Without Cu^{2+} ions, the peak *m/z* 463.2301 corresponded to [**H1** + H]$^+$. When 1.0 equiv. of Cu^{2+} was introduced to the sensor system, a new peak appeared at *m/z* 524.1481 (Fig. S3) and was assigned to the single-charged complex [**H1** – H^+ + Cu^{2+}]$^+$ (the calculated $[C_{26}H_{29}CuN_4O_4]^+$ value was 524.1485), which reflected that Cu^{2+} induced deprotonation in the secondary amine "NH". Isotopic analysis of ESI(+)-MS also supported the above deduction (Fig. S3).

The emission spectra are shown in Fig. 2c. When excited at 464 nm, the fluorescence intensity at 543 nm decreased (~92%) drastically with the sequential addition of Cu^{2+} ions. The fluorescence quenching might come from the paramagnetic and heavy atom quenching effect of Cu^{2+}. The inset of Fig. 2c also demonstrated the formation of the 1:1 **H1**/Cu^{2+} adduct in buffer solution. Along with the developing of the complex **H1**/Cu^{2+}, the Φ_F value decreased from 0.21 to 0.017.[12] The associate constant K_a,[13] derived from the fluorescent titration curve, was $(3.9 \pm 0.28) \times 10^6$ M^{-1}. As shown in Fig. 2d, the fluorescence intensity of **H1** (10 μM) at 543 nm decreased linearly with the concentration of Cu^{2+} (0.0–10.0 μM, R = 0.99966) up to a ratio (**H1**/Cu^{2+}) of 1:1.

Influence of pH on H1/Cu^{2+} adduct and spectral responses

To elucidate the mechanism of recognition, the pH titration was carried out in the presence of 1.0 equiv. Cu^{2+} ion in ethanol-water (1/9, v/v) solution. In the pH range from 1.7 to 4.5, a small increase of intensity in absorption (Fig. 3 and S4) and fluorescence (Fig. 4 and S5) was observed; it also displayed a 13 nm and 12 nm red shift in absorption and fluorescence emission respectively. These slight effects were similar to pH-titration (pH 2~4.5) (Fig. 4, black curve) of free **H1** and signified that no interaction occurred between **H1** and Cu^{2+}, due to the electric charge repulsion between the protonated pyridine (the pyridine was protonated in this acidic

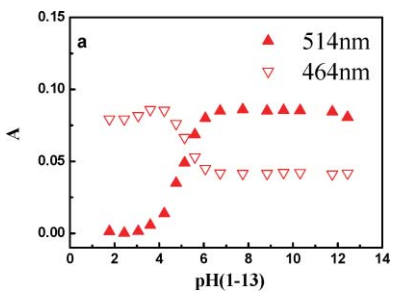


Fig. 3 Dependence of absorbance of the band at 464 nm and 514 nm on pH for a solution of [**H1** + Cu^{2+}] adduct (5 μM **H1** and 5 μM Cu^{2+}) in ethanol-water solutions (1/9, v/v).

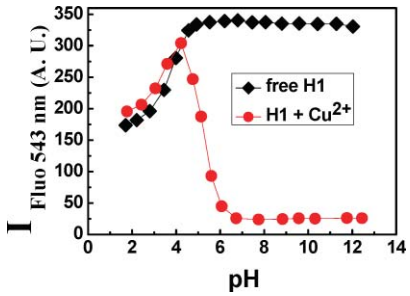
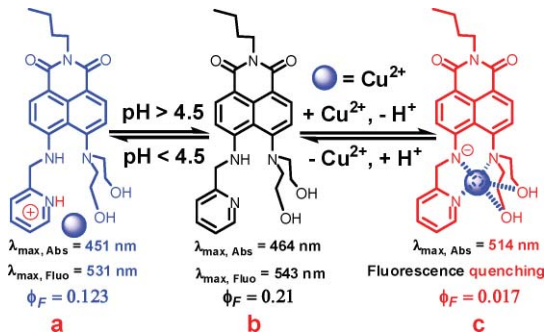


Fig. 4 The pH-titration of free H1 (◆) and **H1** + 1.0 equiv. Cu^{2+} (●) in aqueous ethanol solution (10% ethanol), note: the fluorescence intensity was normalized.

situation) and positively charged Cu^{2+}, which indeed prevented the receptor capturing Cu^{2+} as shown in Scheme 4a–b.



Scheme 4 Influence of pH on the coordination between **H1** and Cu^{2+} and spectra.

With the increase of pH from 4.5 to 6.0, a large red-shift in absorption (Fig. 3 and S4) and a drastic decrease in fluorescence intensity (Fig. 4 and S5) were observed. This indicated that the receptor gradually captured Cu^{2+} and formed an **H1**/Cu^{2+} complex due to the recovery of the coordination ability of the receptor upon releasing the proton in protonated pyridine (protonation in acidic solution) with the increase of pH as shown in Scheme 4b–c.

For **H1** in near neural and basic solution (pH 6–12), the absorption and fluorescence spectra remained unchanged which indicated that the **H1**/Cu^{2+} adduct dominated the equilibrium in such a wide pH range. Moreover, these results also suggested that the **H1**/Cu^{2+} adduct was stable in a strong basic condition (here pH ~12), that is, the OH could not displace the receptor as the ligand to coordinate with Cu^{2+} (commonly, $Cu(OH)_2$ could be formed in basic condition). To further evaluate the pH-independence of **H1** to sense Cu^{2+}. The Cu^{2+}-titration experiment was carried out in strong alkaline solution (pH 11.55) and gave a similar fluorescence profile with that obtained in neutral buffer (Fig. S6). These results clearly demonstrated that sensor **H1** could work in strong basic solution and was a pH-resisted chemosensor. To the best of our knowledge, this is the first fluorochemosensor for Cu^{2+} working over such a wide pH range from 6 to 12.

The responses of H1 to various metal ions

The fluorescence titration of **H1** with various metal ions was conducted to examine the selectivity. As shown in Fig. 5, 6, there was no response to other heavy metal ions and alkaline ions except

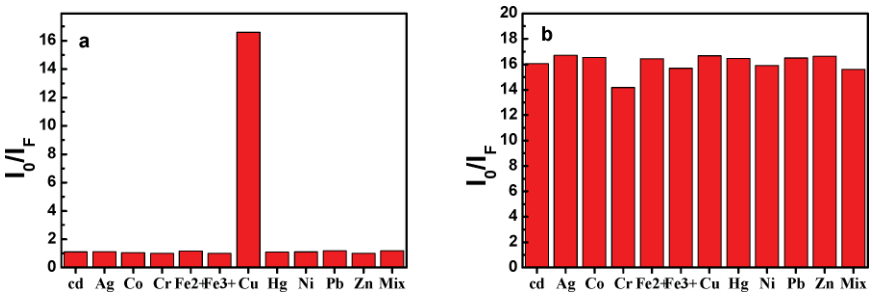


Fig. 5 Fluorescent response of **H1** to various metal ions in HEPES (20 mM, pH 7.05) buffer solution (containing 10% ethanol as the cosolvent). (a) **H1** = 10 μM, Cu^{2+} = 10 μM and concentration of other metal ions was 50 μM. (b) **H1** = 10 μM, Cu^{2+} = 10 μM and other cations were 50 μM.

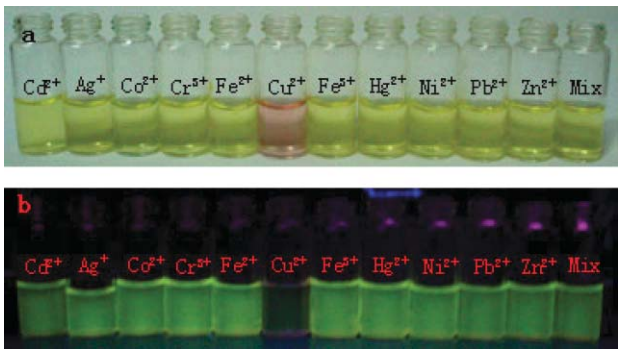


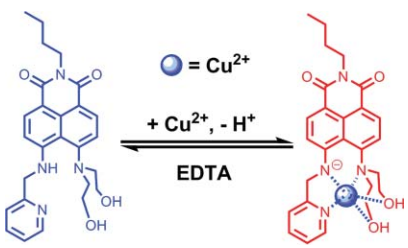
Fig. 6 (a) Color and (b) fluorescent change of **H1** to various metal ions in HEPES (20 mM, pH 7.05) buffer solution (containing 10% ethanol as the cosolvent). **H1** = 10 μM, Cu^{2+} = 10 μM and the concentration of other metal ions was 50 μM.

Cu^{2+} ions. The competition experiments were conducted in the presence of 1.0 equiv. Cu^{2+} ion mixed with 5.0 equiv. other cations. As shown in Fig. 5b, only Cr^{3+} slightly disturbed the intensity rations (I_0/I_F at 543 nm) by comparison with that without the other metal ions besides Cu^{2+}. This means that **H1** had a high selectivity for Cu^{2+}.

The binding mode

For sensor **H1**, two OH groups displaced two pyridine N fragments as the receptor compared with its analogue **Q3**.[4d] As expected, the modification of the receptor obviously improved the selectivity and solubility, which made **H1** work well in aqueous buffer solution.

The UV/vis and fluorescent spectroscopic responses were rationalized by the simplest mechanism as shown in Scheme 5. It has been mentioned above that the red-shift of the main absorption band from 464 nm to 514 nm in the presence of Cu^{2+} resulted from Cu^{2+}-induced deprotonation of the "NH" moiety and the resulting enhancement of electron donation ability.



Scheme 5 The proposed binding mode.

The HR-ESI(+)-MS experiment also confirmed that the Cu^{2+} induced the deprotonation of the "NH" fragment as demonstrated above. In addition, when ETDA was added to the sensing system, the UV/Vis and fluorescent spectra were recovered. The result indicated that the Cu^{2+}-induced deprotonation reaction was reversible. Taking together these results, a plausible binding mode was proposed in Scheme 5, in which Cu^{2+} was coordinated with a pyridine N and two OH groups. A similar coordination mode was reported previously.[4d]

Conclusion

The Cu^{2+}-specific fluorescent sensor **H1** shows a large red-shift in absorption and an on-off fluorescence response in emission in neutral aqueous buffer solution, which made **H1** serve as a naked-eye, dual-channel colorimetric and fluorescent probe for Cu^{2+}. Furthermore, it could work over a wide pH range from 6.0 to 12.0, which was important for use in practical view. To the best of our knowledge, this is the first fluorochemosensor for Cu^{2+} working over such a wide pH range. Based on both the spectral responses and HRES(+)MS analysis, a preliminary mechanism is proposed based on the Cu^{2+}-induced deprotonation of the secondary amine directly conjugating with the 4,5-diamine-1,8-naphthalimide chromophore. These results could promote the application of 4,5-diamino-1,8-naphthalimide (DNP) as chromophore in the design of fluorescent sensors with large colorimetric and ratiometric response.

Acknowledgements

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