A selective and ratiometric Cu^{2+} fluorescent probe based on naphthalimide excimer–monomer switching†

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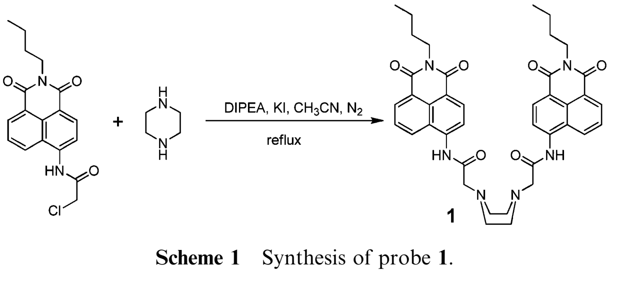
A fluorescent probe was designed and shown to detect Cu^{2+} ratiometrically and selectively in aqueous solutions based on naphthalimide excimer–monomer switching.

Fluorescent probes that convert molecular recognition into highly sensitive and easily detected fluorescence signals have been actively investigated in recent years.[1] In particular, ratiometric measurements have the important feature that they permit signal rationing, and thus increase the dynamic range and provide built-in corrections for environmental effects.[2] The design of fluorescent probes for Cu^{2+} is actively investigated as this metal ion is a significant environmental pollutant and yet also an essential trace element in biological systems.[3] Nevertheless, few ratiometric fluorescent probes for Cu^{2+} have been reported due to the fluorescence quenching nature of paramagnetic Cu^{2+}.[4–8] However, most of the reported probes were only utilized in pure organic solvents. In some cases the Cu^{2+} recognition is interfered by other metal ions such as Ag^+, Hg^{2+} and Pb^{2+}. To achieve Cu^{2+}-only ratiometric sensing in aqueous solution, fluorescent probes require deliberate design.

Ratiometric fluorescent probes involve the observation of changes in the ratio of the intensities of the emission at two wavelengths. So there are two basic requirements to construct a ratiometric fluorescent probe for Cu^{2+}. Firstly, a signalling mechanism is required which can turn the Cu^{2+} recognition event into a ratiometric fluorescence signal. Popular mechanisms include intramolecular charge transfer (ICT),[5] fluorescence resonance energy transfer (FRET),[6] and the pyrene monomer–excimer transformation.[7] Also, some reported ratiometric Cu^{2+} fluorescent probes are based on a chemodosimeter approach through irreversible chemical reactions.[8] Secondly, there is the requirement to protect the fluorescence from being quenched by Cu^{2+}. A simple analogy of the strategies for protecting fluorescence from Cu^{2+} quenching is that of protecting land (the fluorophore) from flooding (with Cu^{2+}). At least three methods are available to accomplish this: (1) build a dam to prevent the water (Cu^{2+}) from gaining close proximity to the land (fluorophore); (2) increase the altitude of the land (fluorophore) in order to prevent flooding by lower level water (Cu^{2+}); and (3) introduce a "channel" to drain the water away.

We have shown that the addition of a carbonyl group between 1,8-naphthalimide and di-2-picolylamine demonstrated the ability to not only block heavy and transition metal (HTM) ions from interacting with the naphthalimide ('dam' strategy) but also increase the oxidation potential of naphthalimide ('altitude' increasing) and to act as a sacrificial donor ('channel' strategy) in order to maintain fluorescence.[9] In order to get a ratiometric fluorescence signal for Cu^{2+} recognition, in this work, two *N*-butyl-4-acetamido-1,8-naphthalimides were linked by a piperazine ring to make probe **1** (Scheme 1). Due to the electron-withdrawing effect of the amide groups, **1** in dichloromethane displays a relatively short emission wavelength with a maximum at 450 nm (Fig. 1). By increasing the polarity of solvents, the piperazine presumably changes from its chair conformation to a boat-like conformation resulting in the switch of the naphthalimide monomer emission (450 nm) to an excimer emission (~550 nm) (Fig. 1).

The fluorescence response of **1** to metal ions was firstly investigated in CH_3CN (Fig. 2a). Addition of HTM ions caused an increase in monomer emission at the expense of the excimer emission, the extent to which depended on the nature of the ion. It was notable that the addition of less than 1 equiv. of metal ions, for example Cu^{2+} titration curves shown in Fig. 2b, did not change the fluorescence emission. However, in the UV-Vis spectra, the first equiv. of Cu^{2+} caused the absorption band to broaden and red-shift with a decrease of the maximum absorption at 370 nm (Fig. 3a). The addition of the second equiv. of Cu^{2+} induced an enhanced blue-shift of the maximum absorption band (Fig. 3b). Since the same absorption spectra of **1** in CH_3CN was observed in CH_2Cl_2 (Fig. S1, ESI†),[7] we hypothesize that free **1** in polar solvents should display a dynamic excimer emission, which results from a naphthalimide dimer formed in the excited state; whereas, the **1**/Cu^{2+} (1 : 1) complex should display a static excimer emission arising from a naphthalimide dimer in the ground state. Further evidence of the formation of the



Scheme 1 Synthesis of probe **1**.

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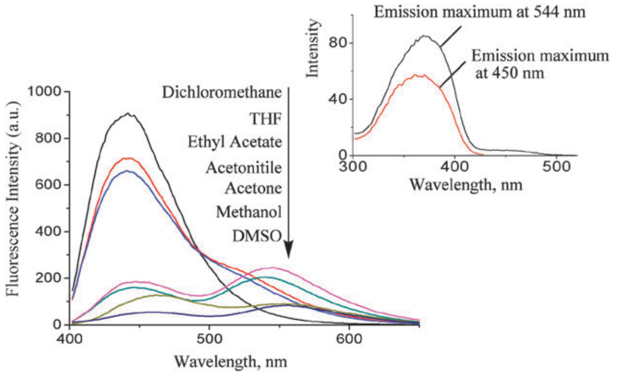


Fig. 1 Fluorescence spectra of 10 μM **1** in different solvents. Inset: fluorescence excitation spectra of **1** in CH_3CN associated with monomer and excimer emissions. The excitation spectra associated with monomer and excimer emissions are characteristically similar.

naphthalimide static excimer of **1** with binding Cu^{2+} is the red-shifted excitation spectrum of the **1**/Cu^{2+} (1 : 1) complex in comparison to that of free **1** (Fig. S2, ESI†).[7]

In order to determine the final stoichiometry of the **1**–Cu^{2+} complex, the method of continuous variations (Job's method) was used (Fig. S3, ESI†). As expected, the result indicated the formation of a **1**/Cu^{2+} (1 : 2) complex showing naphthalimide monomer emission. A proposed mechanism associated with Cu^{2+} binding to **1** is shown in Scheme 2. ^{1}H-NMR analysis provides evidence that metal ions are bound to the amide oxygen of **1** in CH_3CN, resulting in upfield shifts of the resonance of the adjacent NH proton.[10] For example, addition of Zn^{2+} promotes an upfield shift (10.61 to 9.63 ppm) of the resonance of the adjacent NH proton in **1** (Fig. S4, ESI†).

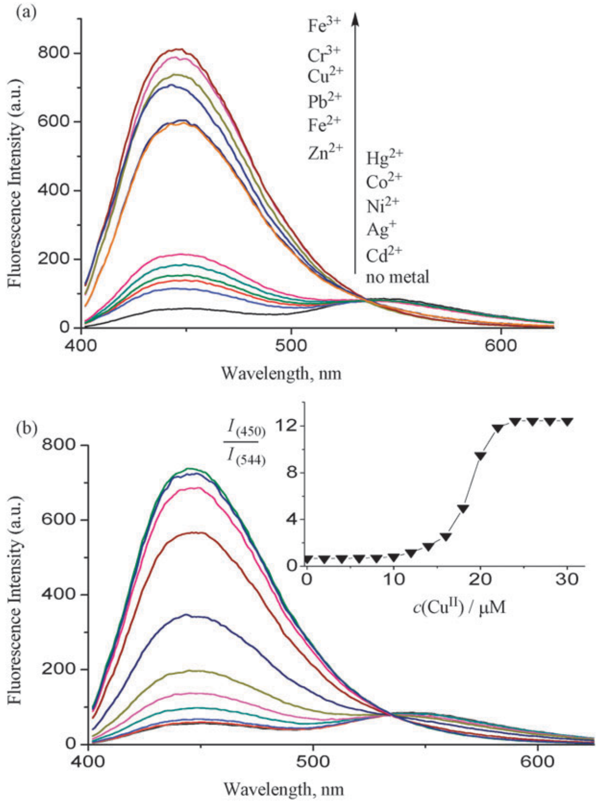


Fig. 2 (a) Fluorescence spectra of 10 μM **1** in the presence of different HTM ions (30 μM) in CH_3CN. (b) Fluorescence spectra of 10 μM **1** in the presence of different concentrations of Cu^{2+} in CH_3CN. Excitation at 370 nm. Inset: the ratiometric calibration curve I_{450}/I_{544} as a function of Cu^{2+} concentration.

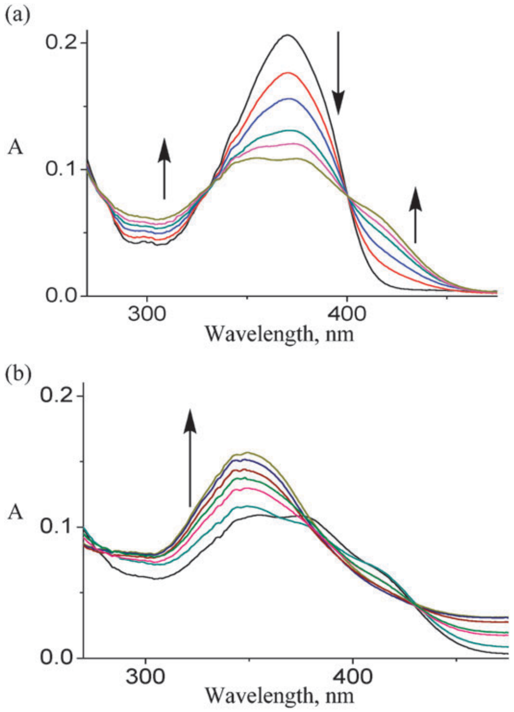
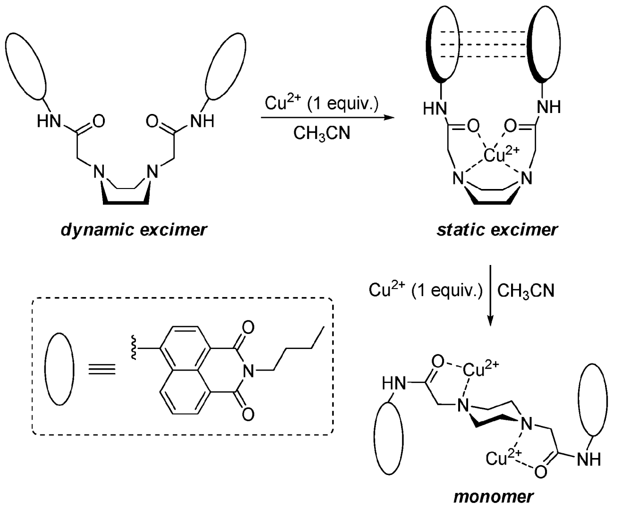


Fig. 3 UV-Vis absorption spectra of 10 μM **1** in the presence of different concentrations of Cu^{2+} in CH_3CN. (a) 0–10 μM; (b) 10–22 μM.

Significantly, the fluorescence responses of **1** to metal ions in aqueous solutions (CH_3CN : HEPES = 50 : 50) were detected (Fig. 4a). As shown in Fig. 4, the addition of Cu^{2+} induced a selective increase in monomer emission. The addition of other metal ions, such as Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, Fe^{2+}, Fe^{3+}, Cr^{3+}, Ag^+, Hg^{2+}, Pb^{2+}, produced a negligible change in the fluorescence spectra of **1**. Therefore, in aqueous solutions, only Cu^{2+} could trigger the excimer–monomer switching of **1**. Fluorescence titration



Scheme 2 Proposed mechanism of stepwise binding mode of **1** with Cu^{2+}.

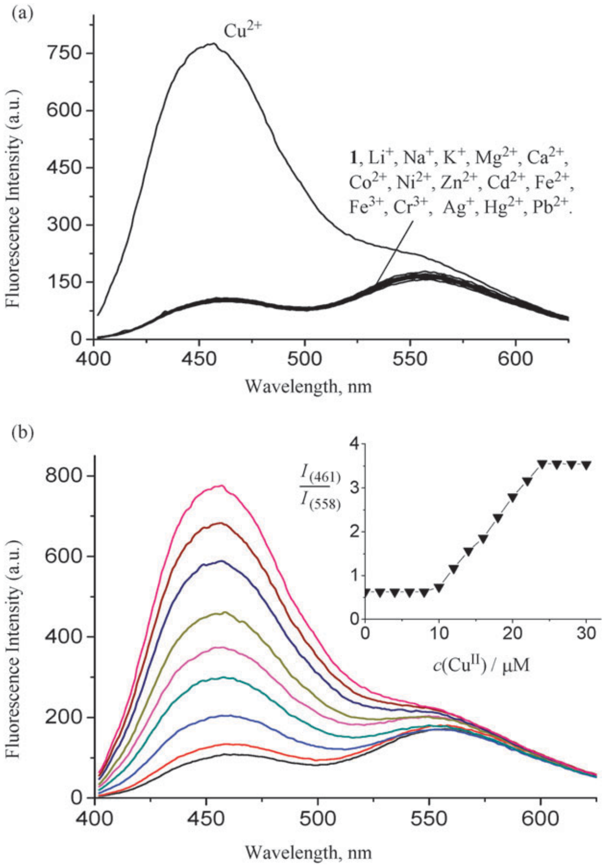


Fig. 4 (a) Fluorescence spectra of 10 μM **1** in the presence of different HTM ions (30 μM) in aqueous solutions (CH_3CN : HEPES = 50 : 50; HEPES, 0.5 M, pH 7.4). (b) Fluorescence spectra of 10 μM **1** in the presence of different concentrations of Cu^{2+} in aqueous solutions (CH_3CN : HEPES = 50 : 50; HEPES, 0.5 M, pH 7.4). Excitation at 370 nm. Inset: ratiometric calibration curve I_{461}/I_{558} as a function of Cu^{2+} concentration.

experiments (Fig. 4b) showed that the association constant (K_a) of **1** with Cu^{2+} is 2.94×10^5 M^{-1} (error < 10%).[11] The inset in Fig. 4b exhibits the dependence of the intensity ratios of emission at 461 nm to that at 558 nm (I_{461}/I_{558}) on Cu^{2+}. This curve can serve as the calibration curve for the detection of Cu^{2+}. These results suggest that **1** is a highly selective and ratiometric fluorescent sensor for Cu^{2+} in aqueous solutions.

In conclusion, probe **1** was designed based on strategies to avoid fluorescence quenching with metal ions, and was shown to detect Cu^{2+} ratiometrically and completely selectively in aqueous solutions based on naphthalimide excimer–monomer switching.

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Notes and references

1 (*a*) S. W. Thomas III, G. D. Joly and T. M. Swager, *Chem. Rev.*, 2007, **107**, 1339; (*b*) R. Martínez-Máňnez and F. Sancenón, *Chem. Rev.*, 2003, **103**, 4419; A. P. de Silva, H. Q. Nimal Gunaratne, T. Gunnlaugsson, A. J. M. Huxley, C. P. McCoy, J. T. Rademacher and T. E. Rice, *Chem. Rev.*, 1997, **97**, 1515; (*c*) H. N. Kim, M. H. Lee, H. J. Kim, J. S. Kim and J. Yoon, *Chem. Soc. Rev.*, 2008, **37**, 1465; (*d*) S. K. Kim, D. H. Lee, J.-I. Hong and J. Yoon, *Acc. Chem. Res.*, 2009, **42**, 23; (*e*) Z. Xu, X. Chen, H. N. Kim and J. Yoon, *Chem. Soc. Rev.*, 2010, **39**, 127.

2 (*a*) A. P. Demchenko, *Lab Chip*, 2005, **5**, 1210; (*b*) J. V. Mello and N. S. Finney, *Angew. Chem., Int. Ed.*, 2001, **40**, 1536; (*c*) Z. Xu, N. J. Singh, J. Lim, J. Pan, H. N. Kim, S. Park, K. S. Kim and J. Yoon, *J. Am. Chem. Soc.*, 2009, **131**, 15528; (*d*) Z. Xu, K. Baek, H. N. Kim, J. Cui, X. Qian, D. R. Spring, I. Shin and J. Yoon, *J. Am. Chem. Soc.*, 2010, **132**, 601.

3 (*a*) E. L. Que, D. W. Domaille and C. J. Chang, *Chem. Rev.*, 2008, **108**, 1517; (*b*) R. Krämer, *Angew. Chem., Int. Ed.*, 1998, **37**, 772; (*c*) G. Li, Z. Xu, C. Chen and Z. Huang, *Chem. Commun.*, 2008, 1774; (*d*) K. M. K. Swamy, S.-K. Ko, S. K. Kwon, H. N. Lee, C. Mao, J.-M. Kim, K.-H. Lee, J. Kim, I. Shin and J. Yoon, *Chem. Commun.*, 2008, 5915; (*e*) X. Chen, M. J. Jou, H. Lee, S. Kou, J. Lim, S.-W. Nam, S. Park, K.-M. Kim and J. Yoon, *Sens. Actuators, B*, 2009, **137**, 597; (*f*) Y. Zhou, F. Wang, Y. Kim, S.-J. Kim and J. Yoon, *Org. Lett.*, 2009, **11**, 4442.

4 M. Royzen, Z. Dai and J. W. Canary, *J. Am. Chem. Soc.*, 2005, **127**, 1612.

5 (*a*) Z. Xu, Y. Xiao, X. Qian, J. Cui and D. Cui, *Org. Lett.*, 2005, **7**, 889; (*b*) Z. Xu, X. Qian and J. Cui, *Org. Lett.*, 2005, **7**, 3029; (*c*) H. Yang, Z. Liu, Z. Zhou, E. Shi and F. Li, *Tetrahedron Lett.*, 2006, **47**, 2911; (*d*) Z. Xu, J. Pan, D. R. Spring, J. Cui and J. Yoon, *Tetrahedron*, 2010, **66**, 1678.

6 H. Kim, S. Park, S. Yoon and J. S. Kim, *Tetrahedron*, 2008, **64**, 1294.

7 (*a*) H. Kim, J. Hong, A. Hong, S. Ham, J. Lee and J. S. Kim, *Org. Lett.*, 2008, **10**, 1963; (*b*) H. Jung, M. Park, D. Han, E. Kim, C. Lee, S. Ham and J. S. Kim, *Org. Lett.*, 2009, **11**, 3378.

8 (*a*) W. Lin, L. Yuan, W. Tan, J. Feng and L. Long, *Chem.–Eur. J.*, 2009, **15**, 1030; (*b*) N. Shao, J. Jin, H. Wang, Y. Zhang, R. Yang and W. Chan, *Anal. Chem.*, 2008, **80**, 3466; (*c*) Y. Xiang and A. Tong, *Luminescence*, 2008, **23**, 28; (*d*) R. Martínez, A. Espinosa, A. Tárraga and P. Molina, *Tetrahedron*, 2008, **64**, 2184.

9 Z. Xu, S. Han, C. Lee, J. Yoon and D. R. Spring, *Chem. Commun.*, 2010, **46**, 1679.

10 A literature which reported the binding of amide oxygen with HTM ions resulting in upfield shifts of the resonance of the adjacent NH proton in ^1H NMR spectra; D. S. Marlin, D. G. Cabrera, D. A. Leigh and A. M. Z. Slawin, *Angew. Chem., Int. Ed.*, 2006, **45**, 77.

11 (*a*) K. A. Conners, *Binding Constants*, Wiley, New York, 1987; (*b*) Association constants were obtained using the computer program ENZFITTER, available from Elsevier-BIOSOFT, 68 Hills Road, Cambridge CB2 1LA, United Kingdom.