Development of off–on fluorescent probes for heavy and transition metal ions†

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A carbonyl group was positioned between 1,8-naphthalimide and di-2-picolylamine (DPA) and played a key role of displaying fluorescence enhancements with heavy and transition metal (HTM) ions through increasing the oxidation potential of the fluorophore, blocking HTM ions from sterically interacting with the naphthalimide fluorophore, and by acting as a sacrificial donor.

Heavy and transition metal (HTM) ions play an important role in many biological and environmental processes.[1] For example, Zn^{2+} and Cu^{2+} play essential roles in biochemical reactions like catalysis, transport or biosynthesis at trace level (<1 μM). Some heavy metal ions, like Cd^{2+} or Hg^{2+}, are potentially carcinogenic or mutagenic and many HTM ions affect the toxicity of organic xenobiotics through interaction with metabolizing enzymes or protein synthesis. Fluorescent probes are powerful tools to monitor *in vitro* and/or *in vivo* HTM ions because of the simplicity and high sensitivity of fluorescence. The fluorescence enhancement (off–on signal) induced by complexation with HTM ions is more desirable than fluorescence quenching (on–off signal) in terms of increased sensitivity and selectivity. However, the development of off–on fluorescent probes for HTM ions remains a significant challenge.[2] A typical fluorescent probe for HTM ions contains a linked fluorophore (the signal source) to a receptor (the recognition site).[3] Unfortunately, fluorescence *quenching* is usually observed when HTM ions are bound to probes of this type due to quenching by heavy atom enhanced intersystem crossing (*e.g.* Hg^{2+})[4] or energy/electron transfer (*e.g.* paramagnetic Cu^{2+}).[5] Herein we present three general strategies for surmounting these hurdles. Moreover, the design and synthesis of a probe based on these strategies is described, which displays a fluorescence enhancement (FE) with HTM ions.

In order to prevent fluorescence quenching and preserve the ability for FE to take place upon binding of the HTM ions, at least three strategies are available. One approach is to prevent the close proximity of the HTM ions to the fluorophore. For example, Ghosh's cryptand receptor blocks HTM ions from sterically and electronically interacting with the peripheral fluorophore.[6a] In a similar manner, the twisted and thus largely decoupled benzene ring in Rurack's system[6b] and the twisted piperazine ring in Xiao's probe[6c] lead to high FE. The second approach is to increase the oxidation potential of the fluorophore. This should reduce the rate of single electron transfer (SET) from its singlet excited state to HTM ions and, thereby, make quenching less efficient.[7] The probe developed by Ramachandram and Samanta relies on this strategy.[7] The third approach is to introduce sacrificial donors that participate in SET with the HTM ions instead of the fluorophore. In previous studies, it was found that two unprotonated aromatic nitrogens act as electron donors to Cu^{2+} to avoid quenching the fluorescence.[8]

In this report, we designed a new sub-class of probe by combining 1,8-naphthalimide (fluorophore) with di-2-picolylamine (DPA, receptor), which utilized photoinduced electron transfer (PET) as the mechanism for signal induction (Fig. 1). It was found that HTM ions quenched fluorescence of **1** in acetonitrile (Fig. 2) and aqueous solution (CH_3CN–HEPES = 1 : 9, HEPES 0.5 M, pH = 7.4) (Fig. S1, ESI†).[9] In order to block quenching of the 1,8-naphthalimide singlet excited state by bound HTM ions, inspired by the role it plays in the spirolactam ring-opening process of rhodamine-based probes,[10] a carbonyl group was positioned between the naphthalimide and DPA to generate probe **2**. The electron-withdrawing carbonyl increases the oxidation potential of the fluorophore. In addition, the carbonyl oxygen can coordinate HTM ions along with DPA and then may block HTM ions from sterically interacting with the fluorophore and play the role of a sacrificial donor. Also, the amide linkage can increase the inflexibility of the receptor.[11] The fluorescence spectrum of **2** in acetonitrile contains an emission band with a maximum at 481 nm. The presence of the amide group in **2** decreases the electron donating ability of the conjugated NH nitrogen and therefore results in *ca.* 40 nm blue shift in emission compared to that of **1** (520 nm).[12] Addition of HTM ions to acetonitrile solutions of **2** causes an increase in fluorescence intensity due to PET, the extent to which depends on the nature of the ions, and a shift in the emission maximum from 481 to *ca.* 430 nm due to internal charge transfer (ICT) (Table 1 and Fig. S2–S11, ESI†). The dependence of the ratio of intensities of emission at 430 nm to that at 481 nm (I_{430}/I_{481}) on the nature of the HTM ions indicates that the stoichiometry of **2**–M^{n+} complexes is 1 : 1. The binding of HTM ions to the carbonyl oxygen causes a further decrease in the electron donating ability and a blue shift in the emission maximum (Fig. S2–S11, ESI†).[12] Thus we

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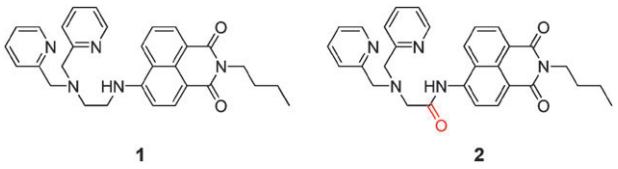

Fig. 1 Structures of the fluorescent probes studied.

can see that the introduction of the carbonyl group in **2** protects fluorescence from quenching by HTM ions. The blue shift in emission can provide a ratiometric fluorescence assay for HTM ions.

Cu^{2+} is the most notorious fluorescence quencher among HTM ions, therefore the study of the **2**–Cu^{2+} complex would representatively elucidate the function of the carbonyl group. Green crystals of **2**–$CuCl_2$ were obtained by vapor diffusion of ether into the CH_3CN solution of **2**–$CuCl_2$ (1 : 1). The single crystal structure of **2**–Cu^{2+} showed that the amide oxygen cooperates with the DPA as a receptor to bind Cu^{2+} (Fig. 3). The carbonyl group keeps Cu^{2+} away from the naphthalimide fluorophore. Table 2 shows the selected bond distances for the crystal of **2**–Cu^{2+}.‡

The emission spectra of **2** and the effects of added Cu^{2+} are shown in Fig. 4. The blue shift in emission is due to the Cu–O bond formation, which increases the electron-withdrawing ability of the carbonyl group. ^{1}H-NMR analysis provides evidence that other HTM ions are also bound to the amide oxygen of **2** in acetonitrile, resulting in upfield shifts of the resonance of the adjacent NH proton.[13] For example, addition of 1 equiv. of Zn^{2+} or Cd^{2+} promotes a large upfield shift (11.72 to 9.73 and 9.49 ppm, respectively) of the resonance of the adjacent NH proton in **2** (Fig. S12 and 13, ESI†). Correspondingly, the absorption maximum of **2** undergoes a blue shift from 371 nm to 348 nm upon addition of both Zn^{2+} and Cd^{2+} due to ICT (Fig. S14 and 15, ESI†).

To further explore the function of the carbonyl group in **2**, fluorescence responses to HTM ions in different ratios of water in acetonitrile were examined. With changes in water percentages between 10 to 80%, **2** exhibited a blue shift and increased intensity of its fluorescence response to Cu^{2+} (Fig. 5). As the water percentage increases beyond 80%, the

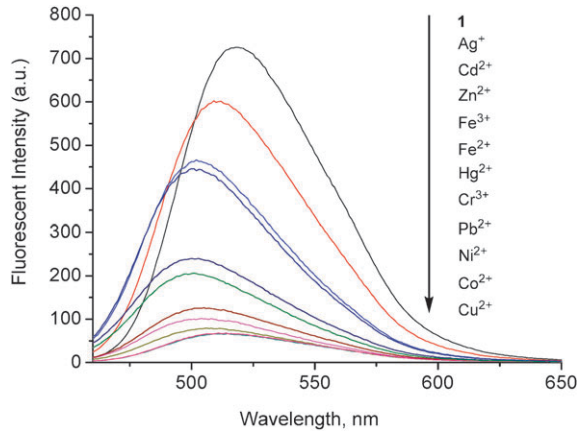

Fig. 2 Fluorescence spectra of **1** in the presence of different HTM ions in CH_3CN. Excitation at 450 nm. [**1**] = 10 μM, [HTM] = 30 μM.

Table 1 Maximum of fluorescence enhancement (FE) and emission wavelength changes of **2** in the presence of HTM ions in acetonitrile[a]

	λ_{em}/nm	FE[b]		λ_{em}/nm	FE
None	481	1	Cu^{2+}	430	3.8
Cr^{3+}	440	58	Zn^{2+}	430	46
Fe^{2+}	427	2.7	Ag^{+}	446	1.6
Fe^{3+}	430	7.6	Cd^{2+}	430	36
Co^{2+}	430	2.8	Hg^{2+}	435	82
Ni^{2+}	435	3.1	Pb^{2+}	427	31

[a] Experimental conditions: [**2**] = 10 μM, [M^{n+}] = 30 μM, λ_{ex} = 360 nm at 25 °C. [b] Relative quantum yield (Φ_F) in comparison to **2** in the absence of HTM ions. Calculated by comparison of corrected spectrum with that of *N*-butyl-4-butylamino-1,8-naphthalimide (Φ_F = 0.81 in absolute ethanol), taking the area under the total emission. The Φ_F of **2** is 0.0026.

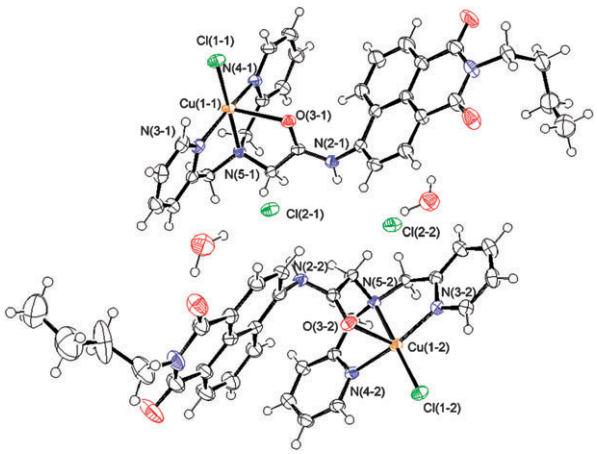

Fig. 3 ORTEP diagram (50% probability ellipsoids) of the **2**–Cu^{2+} complex. The crystal was grown in CH_3CN–Et_2O (1 : 1) with $CuCl_2$.

Table 2 Selected bond distances (Å) for the **2**–Cu^{2+} complex

Cu(1-1)–Cl(1-1)	2.245(8)	Cu(1-2)–Cl(1-2)	2.253(8)
Cu(1-1)–O(3-1)	2.316(2)	Cu(1-2)–O(3-2)	2.305(2)
Cu(1-1)–N(3-1)	1.996(2)	Cu(1-2)–N(3-2)	2.007(2)
Cu(1-1)–N(4-1)	2.010(2)	Cu(1-2)–N(4-2)	2.011(2)
Cu(1-1)–N(5-1)	2.058(2)	Cu(1-2)–N(5-2)	2.058(2)

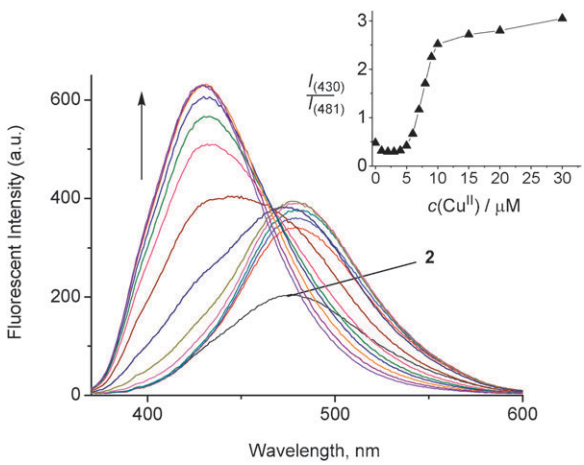

Fig. 4 Fluorescence spectra of 10 μM **2** in the presence of different concentrations of Cu^{2+} in CH_3CN (Ex: 360 nm). Inset: ratiometric calibration curve of I_{430}/I_{481} as a function of Cu^{2+} concentration.

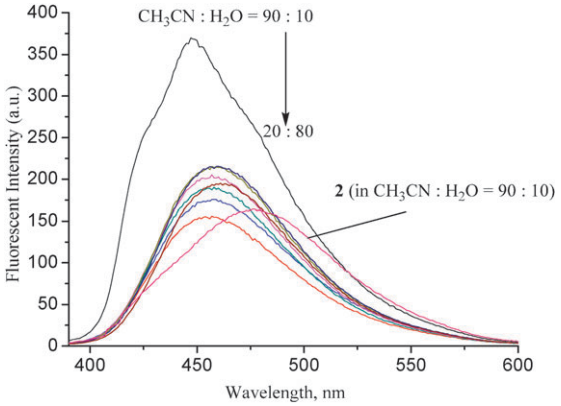

Fig. 5 Fluorescence spectra of 10 μM **2** in the presence of 30 μM Cu^{2+} in acetonitrile–water with different volume ratios.

emission wavelength shift and FE decrease. These phenomena are likely to be a consequence of the effect of water on decreasing interactions between carbonyl oxygen and Cu^{2+} in a manner that is similar to that found in an example described by Kim *et al.*,[14] where a sulfonyl oxygen played a similar role to the carbonyl. In Kim's report, the binding of Cu^{2+} to the sulfonamide group in H_2O–CH_3CN (1 : 1, v/v) induces the formation of an intermolecular pyrenyl static excimer exhibiting an emission at 455 nm along with a weak monomer emission at 375 nm. With the increase of water percentages, the pyrene excimer emission decreased and finally disappeared ($H_2O\%$ > 70%), along with the enhancement of the pyrene monomer emission. In the latest case, Kim *et al.* exchanged the sulfonamide group with an amide group.[15] The coordination of Cu^{2+} by amide oxygen in CH_3CN again induces a pyrene excimer emission.

In conclusion, we have presented strategies for the design of turn-on fluorescent probes for HTM ions. The strategies were used to design a novel probe **2** that undergoes FE in the presence of different HTM ions and a *ca.* 50 nm blue shift in emission in acetonitrile that is suitable for a ratiometric assay. The results suggest that the carbonyl group in **2** increases the oxidation potential of the fluorophore compared to **1**, blocks HTM ions from sterically interacting with the naphthalimide fluorophore, and that it acts as a sacrificial donor. The observations made in this effort should serve as the foundation for the design of new off–on probes for HTM ions.

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Notes and references

‡ Crystal data for the **2**–Cu^{2+} complex: $C_{30}H_{31}Cl_2CuN_5O_4$, M_w = 660.04, prism 0.42 × 0.21 × 0.12 mm, monoclinic $P2_1/n$ (no. 14), a = 27.4477(3), b = 6.6081(1), c = 35.2482(4) Å, α = 90°, β = 112.053(1)°, γ = 90°, V = 5925.47(13) Å^3, Z = 8, T = 180(2) K, D_{calc} = 1.480 g cm^{-3}, λ = 0.71073 Å, μ = 0.962 mm^{-1}, Nonius Kappa CCD diffractometer, 3.53° < θ < 26.02°, 33 837 measured reflections, 11 470 independent (R_{int} = 0.0484), 8556 with $I > 2\sigma(I)$. The structure was solved by direct methods (SHELXS-97) and refined by least squares (SHELXL-97)[16] using Chebyshev weights on F_o^2 to R_1 = 0.040, wR_2 = 0.097 [$I > 2\sigma(I)$], 772 parameters. The water hydrogen atoms were located and their positions were refined satisfactorily, all other hydrogen atoms in calculated positions; goodness-of-fit on F^2 1.04; residual electron density 0.67 e Å^{-3}.

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