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Silver(I) ion detection in aqueous media based on "off-on" fluorescent probe†

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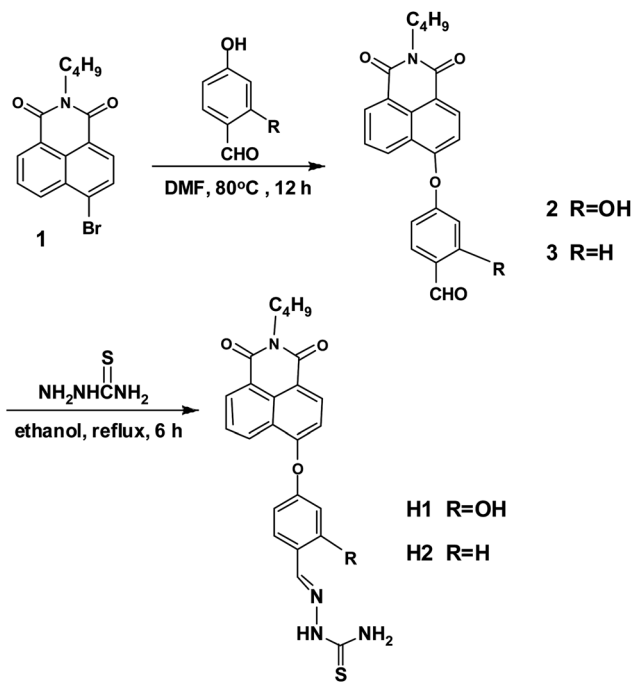
A novel and easily available fluorescent probe bearing naphthalimide and thiosemicarbazide groups has been designed. The probe H1 displays high selectivity and sensitivity to Ag^+ over other metal ions in aqueous media.

Heavy and transition metal ions (HTM) play an essential role in many biological and environmental processes.[1] Among such biologically important metal ions, the silver ion (Ag^+) has long received considerable attention because of its antimicrobial activities. It is believed that Ag^+ can inactivate sulfhydryl enzymes and combine with amine, imidazole, and carboxyl groups of various metabolites.[2] Thus, the development of selective and sensitive methods capable of monitoring Ag^+ is driven by impact on the environment and to humans. Although atomic absorption, plasma emission spectroscopy and anodic stripping voltammetric and potentiometric methods based on ion selective electrodes are used in the quantitative determination of Ag^+,[3] there are considerable advantages of fluorescence methods in terms of sensitivity, speed, simplicity, and non-destructive nature,[4] so fluorescence detection is of particular interest for Ag^+ sensing. In particular, the design of probes that give fluorescent enhancement upon Ag^+ binding remains a significant challenge due to fluorescence quenching by the Ag^+ enhanced inter-system crossing nature and from electron transfer.[5] Reported fluorescent probes for Ag^+ widely use signaling mechanisms of photoinduced electron/energy transfer (PET),[5e] excimer/exciplex formation,[5b,5d,5f] intramolecular charge transfer (ICT),[5c] twisted intramolecular charge transfer (TICT) and Ag^+ promoted spirolactum ring opening of rhodamine probe,[5h,6] *etc*.

C=N isomerization has been applied to design fluorescent probes for the detection of various metal ions,[7] *etc*. It was found that C=N isomerization is the predominant decay process of excited states in compounds with unbridged C=N structure, which usually results in nonfluorescence of those compounds. In contrast, it is reasonable to amplify fluorescence upon binding with metal ions to an elaborately designed molecule framework by blocking C=N isomerization rather than by covalent bridging of the C=N bond. With this in mind, we can reasonably expect that C=N isomerization may also be inhibited by Ag^+ complexation to the recognition moiety linked to the fluorophore.

The design rationale for Ag^+ probe is schematically illustrated in Scheme 1 and is explained as follows. (1) Naphthalimide derivatives act as fluorophores, which have a strong absorption band in the visible region, emit at long wavelengths with large Stokes shifts, possess high fluorescence quantum yields and high photophysical stability.[8] (2) In designing probes, the recognition moiety should be preliminarily considered because they are responsible for the selectivity and binding efficiency of the whole probes. Thiosemicarbazide motif with S and N donor atoms established high affinity for thio- or aminophilic metal ions such as Ag^+.[9] (3) The C=N group is chosen to not only take advantage of C=N isomerization, but also introduces PET phenomenon in fluorescence. Meanwhile, the chelating group C=N demonstrates a high affinity to transition and post-transition metal ions, but less binding affinity toward alkali metal and alkaline



Scheme 1 Synthesis route of **H1** and **H2**.

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earth metal ions due to the difference of electronic structures.[7] Therefore, it is anticipated that selective fluorescence "turn-on" probe for Ag^+ can be established with naphthalimide derivative **H1**.

Compound **1** was synthesized following the reported literature.[10] **H1** and contrast compound **H2** were synthesized from naphthalimide by three-steps reaction (Scheme 1). The structures of **2**, **3**, **H1** and **H2** were characterized by ^1H NMR, ^{13}C NMR and MS spectra (see ESI Fig. S1–S12†).

The photophysical properties of **H1** were carried out at pH 6.5 in ethanol–water solution (4 : 1, v/v, 50 mM HEPES, Fig. S13†). No significant changes in fluorescence spectra are observed when the probe **H1** was exposed to other metal ions due to rapid isomerization of the C=N double bond in the excited state,[7a,7b] although other mechanisms such as photoinduced electron transfer (PET) may also contribute to this.[11] Notably, by adding Ag^+, the fluorescence character of **H1** is different from free **H1** and other metal ions including Na^+, K^+, Ca^{2+}, Mg^{2+}, Zn^{2+}, Pb^{2+}, Cu^{2+}, Hg^{2+}, Co^{2+}, Cd^{2+}, Ni^{2+}, Mn^{2+}, Fe^{3+}, Cr^{3+} (Fig. 1a), its fluorescence can be turned from "off" to "on", resulting in about a 60-fold enhancement of fluorescence at 540 nm. Two emission peaks (one in the blue region λ_{em} 428 nm and one in the red region λ_{em} 540 nm) exhibit optical transduction due to Ag^+ coordination. The enhancement of fluorescence at λ_{em} 428 nm is likely due to restriction of acyclic C=N isomerization in the Schiff base upon addition of Ag^+;[7] the band at 540 nm is due to the naphthalimide and the increase in the fluorescence intensity can be attributed to the PET process being inhibited upon Ag^+ binding to the thiosemicarbazide group.[7c,7d]

To validate the selectivity of probe H1 in practice, the possible interference from other metal ions was assessed through competition experiments. The fluorescence changes of probe **H1** were measured by addition of 1 equiv. of Ag^+ to the aqueous solutions in the presence of 5.0 equiv. of other metal ions (Fig. 1b). Fortunately, all competitive metal ions had no obvious interference with the detection of Ag^+. This result showed that probe **H1** displayed a high selectivity for Ag^+ in this system.

Fluorescence titration of probe **H1** with Ag^+ was then performed. As illustrated in Fig. 2, with the increasing of Ag^+ concentration, a significant increase of the fluorescence intensity at $\lambda_{428\ nm}$ and at $\lambda_{540\ nm}$ was observed, which indicated a ratiometric fluorescence change. The ratio of fluorescence intensity at 540–428 nm increased linearly with the increase of Ag^+ concentration (0.5–9 μM), and the corresponding detection limit of 0.29 μM was also obtained (based on $3 \times \delta_{blank}/k$, where δ_{blank} is the standard deviation of the blank solution and k is the slope of the calibration plot). The present method was applied to determine Ag^+ in tap water. Table **1**† showed the recoveries were between 90% and 120%, thus indicating that the potential application of this proposed method shows high accuracy and good reliability for real water sample analysis.

Job's plot and Benesi–Hildbrand plot experiments were carried out, and the results indicated a 1 : 1 stoichiometry complexation between **H1** and Ag^+ (Fig. S14–S15†). From this, the association constant (k_a) was calculated to be $4.8 \times 10^4\ M^{-1}$ for the **H1**–Ag^+ complex.[12]

To explore the binding mode of compound **H1** with Ag^+, ^1H NMR spectra of compound **H1** and its complex with Ag^+ in DMSO-d_6 were measured as displayed in Fig. 3a. The active protons are assigned as referring to ^1H NMR of compound **H1**. Apparently, the addition of

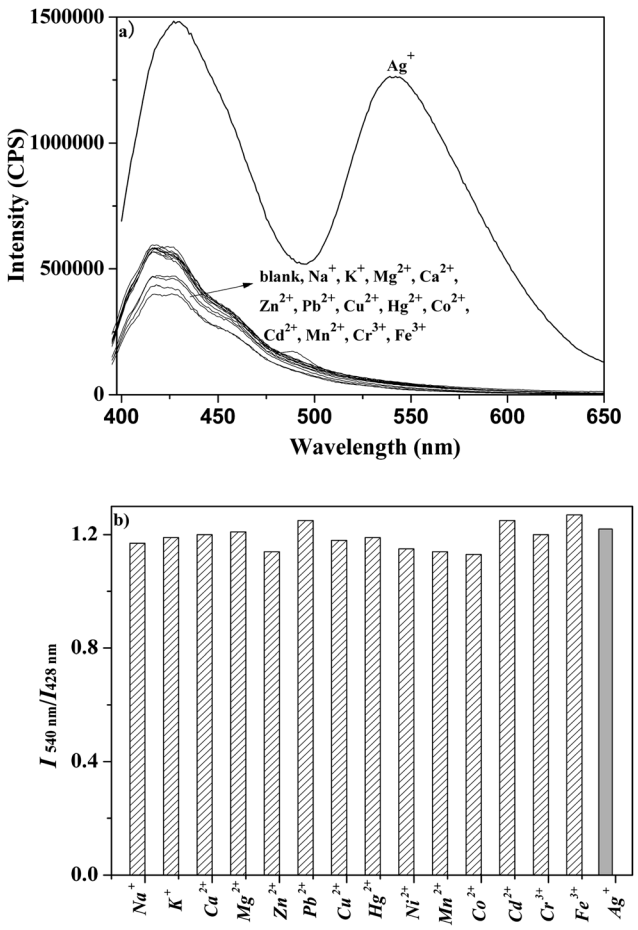

Fig. 1 (a) Fluorescence spectra of 10 μM of **H1** in the presence of 50 μM of various metal ions at pH 6.5 in ethanol–water solution (4 : 1, v/v, 50 mM HEPES). (b) Fluorescence response of 10 μM of **H1** to 10 μM of Ag^+ and to the mixture of 50 μM of individual other metal ions with 10 μM of Ag^+.

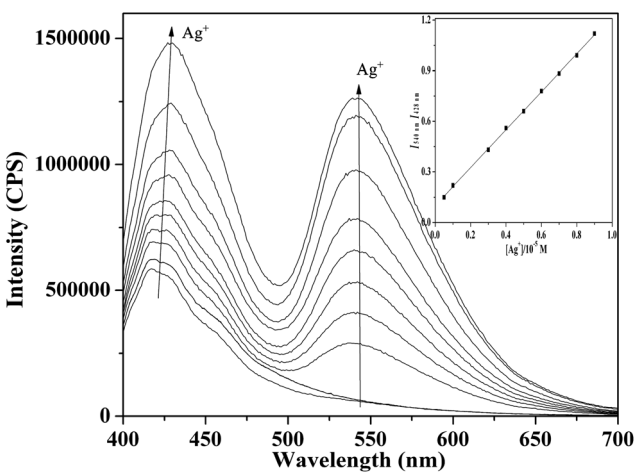

Fig. 2 Fluorescence response of 10 μM of **H1** with various concentrations of Ag^+ at pH 6.5 in ethanol–water solution (4 : 1, v/v, 50 mM HEPES). Inset: rationmetric calibration curve of $I_{540\ nm}/I_{428\ nm}$ as a function of Ag^+ concentrations.

Ag^+ into the solution of **H1** led to an apparent downfield shift of the signals of –OH, S=CH–N–, –CH=N– and $–NH_2$ in certain degrees by *ca.* 0.06 ppm, associated with the enhancement of signal intensity in the **H1**–Ag^+ complex, while the signals of other protons remained nearly unchanged. These changes indicated the electron shielding effect of Ag^+ on protons in its proximity. Accordingly, the structure of **H1**–Ag^+ complex is proposed as shown in Fig. 3b, in which Ag^+ coordinates with thiocarbonyl, phenolic hydroxyl, and Schiff base.

To further gain insight into the proposed complexation mode, and to evaluate the role of oxygen atom of phenolic group in probe **H1** which played in the interaction process with Ag^+, compound **H2** (inset Fig. 4) was synthesized. It is very similar to **H1** in structure except for lack of phenolic group. Fig. 4 shows the fluorescence spectra of compound **H2** at pH 6.5 in ethanol–water solution (4 : 1, v/v, 50 mM HEPES) in the presence of above mentioned different metal ions. The emission of **H2** peaked at 430 nm had no response in the presence of Cu^{2+}, Hg^{2+}, Pb^{2+}, *etc.* Meanwhile, no obvious fluorescence enhancement at 430 nm and an emission band with a maximum at 540 nm were observed upon addition of Ag^+ as found as compound **H1**. It is probably because the C=N bond is not obviously included in the formed chelating complex as is crucial role in the complexation with Ag^+.

In conclusion, we have developed a new fluorescent probe for Ag^+ to induce an enhanced fluorescence change. It displayed a highly selective and sensitive fluorescence signal for Ag^+ presumably *via* conformational restriction and the PET process. Moreover, the molecular structure makes it possible to detect trace Ag^+ ratiometrically. The design strategy and remarkable photophysical properties of the probe can extend the concept towards the construction of a new class of fluorescent probes with practical applications for heavy- and transition metal ions. Further studies to include the design of new analogues of **H1** with good solubility in water, are in progress.

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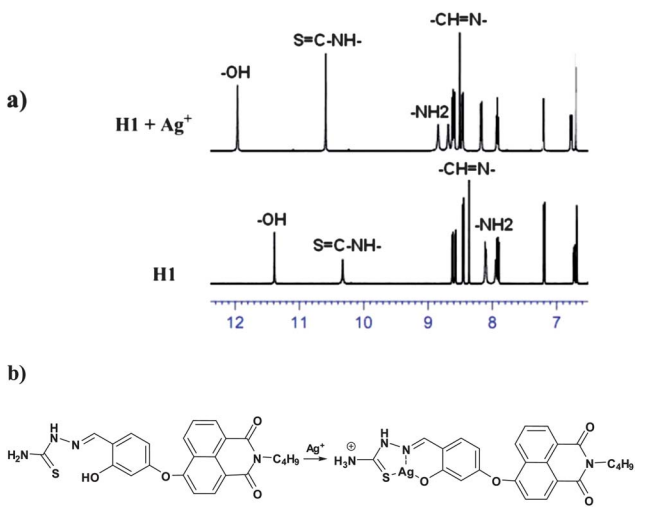

Fig. 3 (a) Partial ^{1}H NMR spectra of **H1** and **H1**–Ag^+. (b) A proposed mode for **H1**–Ag^+.

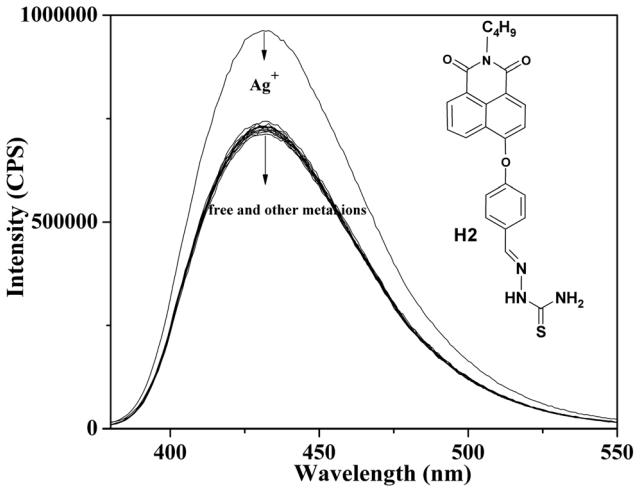

Fig. 4 Fluorescence spectra of **H2** (10 μM) at pH 6.5 in ethanol–water solution (4 : 1, v/v, 50 mM HEPES) upon addition of different metal cations (each concentration was 50 μM) with an excitation wavelength of 360 nm. Inset: molecular structure of **H2**.

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