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A novel fluorescence “on–off–on” chemosensor for Hg^{2+} via a water-assistant blocking heavy atom effect†

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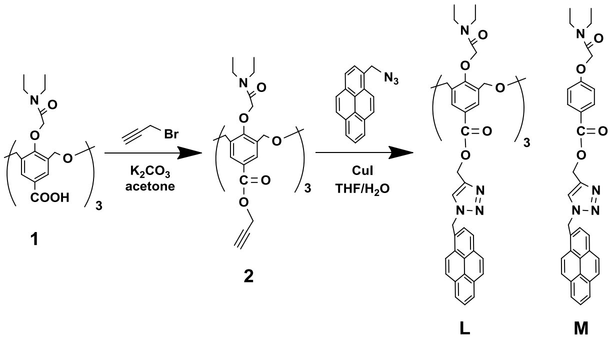
A pyrenyl-appended hexahomotrioxacalix[3]arene L was synthesized as a highly selective “turn off” fluorescence chemosensor for Hg^{2+} in CH_3CN. Within the same system, further coordination of water molecule blocked the heavy atom effect of Hg^{2+} and resulted in the enhancement of fluorescence emission.

Heavy and transition metal (HTM) ions play an important role in living systems and have an extremely toxic impact on the environment.[1] Fluorescent chemosensors are powerful tools to monitor *in vitro* and/or *in vivo* HTM ions because of the simplicity and high sensitivity of fluorescence.[2] However, fluorescence quenching is usually observed when HTM ions are bound to sensors due to the heavy atom enhanced intersystem crossing (e.g. Hg^{2+})[3] or energy/electron transfer (e.g. paramagnetic Cu^{2+}).[4] Fluorescence quenching is not only disadvantageous for a high signal output upon recognition but also hampers temporal separation of spectrally similar complexes with time-resolved fluorometry.[5] Thus, the fluorescence enhancement induced by complexation with HTM ions is highly desirable in the field of fluorescent chemosensor research.

Since McClure (1952)[3] and Wehry (1972)[4] demonstrated the principle of heavy atom effect quenching on fluorescence emission, over the past several decades, the literature reports focusing on the “Why” of the intrinsic quenching behaviour of HTM ions,[6] but not providing the “How” to block the quenching effect. Therefore, one might consider is whether the heavy atom effect caused by HTM ions can be blocked or indeed avoided? Undoubtedly, if so, it may open up a new strategy to develop new fluorescence turn on sensors for HTM ions.

In 2010, Xu *et al.* presented three general strategies to prevent fluorescence quenching and preserve the ability for fluorescence enhancement to take place upon binding of the HTM ions: (i) preventing the close proximity of the HTM ions to the fluorophore; (ii) increasing the oxidation potential of the fluorophore; (iii) introducing sacrificial donors that participate in single electron transfer with the HTM ions instead of the fluorophore.[7] Recently, we reported the first evidence of the blocking heavy atom effect via a water-assisted and time-dependent fluorescence enhancement detection of Hg^{2+}.[8] In connection with our continuing interest in the development of chemosensors for HTM ions, we herein report the synthesis and sensing properties of a new pyrenyl-appended hexahomotrioxacalix[3]arene chemosensor **L**, which exhibited a “on–off–on” fluorescence response toward Hg^{2+} via a water-assistant blocking heavy atom effect. The present studies also provided another evidence for the blocking heavy atom effect.

The synthetic route of the current sensor is depicted in Scheme 1. Compound **2** was synthesized by the reaction of hexahomotrioxacalix[3]arene (**1**)[9] with propargyl bromide in acetone in the presence of K_2CO_3. The Cu^I-catalyzed 1,3-dipolar cycloaddition reaction of compound **2** with 1-azidomethylpyrene under Click conditions afforded the chemosensor **L** in 66 % yield. Reference compound **M** was synthesized in 72 % yield by using a method similar to that used in the preparation of **L**. All compounds synthesized in this work were fully characterized by spectroscopic



Scheme 1. Synthesis of chemosensor **L** and reference compound **M**.

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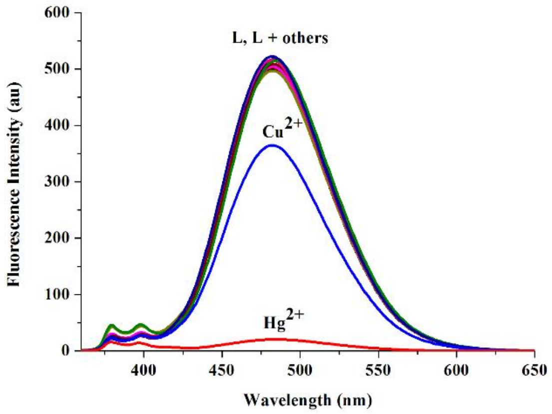

Fig. 1 Fluorescence spectra of **L** (1.0 μM) on addition of various metal cations in CH_3CN, λ_{ex} = 343 nm.

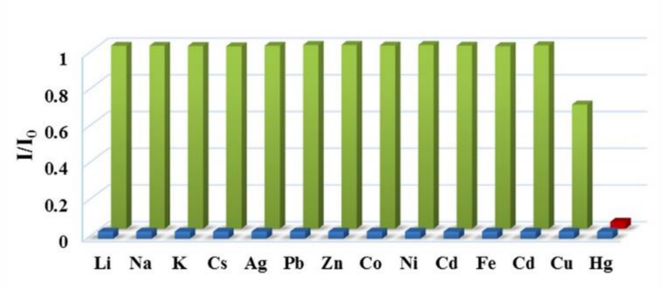

Fig. 2 Fluorescence response of **L** (1.0 μM) to 10 equiv. of metal cations (the green bar) and to the mixture of 10 equiv. of other metal ions with Hg^{2+} (the blue bar) and the fluorescence response of **L** toward Hg^{2+} + all metal ions (the red bar). I_0 is fluorescent emission intensity at 484 nm of free **L**, and I is the fluorescent intensity after adding metal cations.

data including ^1H, ^{13}C NMR and HRMS (details in ESI†).

The fluorescence properties of **L** and **M** were investigated in CH_3CN (Fig. S1, ESI†). On excitation at 343 nm, the maximum absorption wavelength of the pyrene, **L** displays very weak monomer emission (379 nm and 398 nm) but strong excimer emission (around 484 nm), whereas reference compound **M** exhibit only the monomer band at 379 nm and 398 nm. The excimer emission band was attributed to the interaction of the neighboring pyrene units forming an intramolecular π–π stacking, which was fixed by the oxacalix[3]arene scaffold. To explore the potential application of **L** as chemosensor for the detection of HTM ions, the fluorescence response behaviours of **L** in CH_3CN toward several common metal cations, including Li^+, Na^+, K^+, Cs^+, Ag^+, Cu^{2+}, Pb^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+}, Cr^{3+}, Fe^{3+}, Cd^{2+} and Hg^{2+} by using their perchlorate salts (10 equiv.) was investigated. The fluorescence changes are depicted in Fig. 1, addition of Hg^{2+} led to significant fluorescence quenching of up to 96 %. Cu^{2+} ions also resulted in quenching (32 %) but of much smaller scale than Hg^{2+}. Moreover, upon interaction with other metal cations, a much weaker response is given compared to Hg^{2+}. To evaluate the specific fluorescence response toward Hg^{2+}, a group of metal cations and an ion mixture were chosen as the interferential ions to perform the fluorescence tests (Fig. 2). Except for Cu^{2+}, it was found that the rest of the metal cations exerted a negligible or very small influence on the fluorescence spectra of **L**. Thus, **L** can be used as a Hg^{2+} selective fluorescent chemosensor in the presence of most competing metal cations.

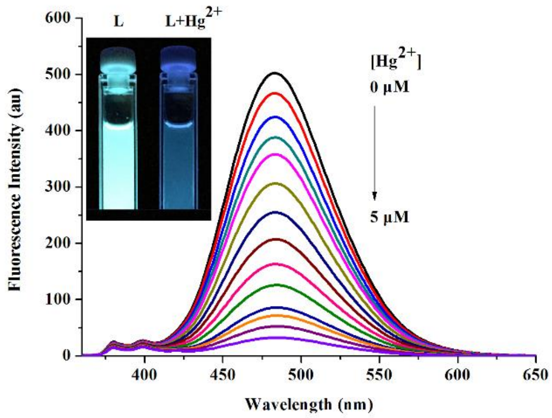

Fig. 3 Fluorescence spectra of **L** (1.0 μM) upon the addition of increasing amount of Hg^{2+} in CH_3CN. λ_{ex} = 343 nm. The inset shows the fluorescence colour of **L** before and after the addition of Hg^{2+}.

To demonstrate the ability of **L** as fluorescent sensor for Hg^{2+}, we performed the fluorescence quenching titration with Hg^{2+} in CH_3CN (Fig. 3). The titration profiles revealed that the initial emission intensity of **L** gradually quenched upon the addition of increasing amounts of Hg^{2+}. The addition of 5 equiv. of Hg^{2+} to the solution of **L** resulted in both the monomer and excimer emission becoming quenched due to the heavy atom effect imparted by Hg^{2+}, which increases the probability for intersystem crossing to the triplet state because of large spin-orbit coupling contribution.[10] A good linear relationship was observed between the emission intensities and concentration of Hg^{2+}, and the limit of detection (LOD) was found to be 3.48×10^{-8} M based on the formula LOD = $3\sigma/K$ (Fig. S2, ESI†).[11] The detection limits were sufficiently low to detect the submillimolar concentrations of Hg^{2+} ion found in many chemical and biological systems. The Job's method monitored by fluorescence intensities was applied to examine the stoichiometry of the **L**/Hg^{2+} complex, indicating a 1:1 stoichiometry of **L** to Hg^{2+} in the complex (Fig. S3, ESI†). The association constant (K_a) was calculated to be 3.3×10^5 M^{-1} according to the spectral titration data using the Benesi–Hildebrand equation (Fig. S4, ESI†).[12] These results indicated that **L** has an excellent sensitivity toward Hg^{2+} with a detection limit in the nM level, and even was applicable for quantitative detection of Hg^{2+}.

For practical applications, the effect of water as a solvent on the detection of Hg^{2+} was also monitored. To study the selective detection of Hg^{2+} from aqueous samples, the fluorescence response of **L** (in CH_3CN) upon addition of water was initially monitored. The addition of water showed a negligible effect on fluorescence intensity and the spectrum remains unaffected even after 1 h (Fig. S5, ESI†). Consequently, we further checked the selectivity of **L** for Hg^{2+} in a H_2O–CH_3CN (v/v, 1:10) mixed solvent. Unexpectedly, upon the addition of Hg^{2+} to the mixed solvent system, the fluorescence spectrum does not show any significant change. Motivated by these results, we expanded this study in order to systematically investigate the role of the water molecules in the **L**/Hg^{2+} complex. In a specially designed experiment, 5 equiv. of

Hg^{2+} was added to quenched the fluorescence emission of **L** in CH_3CN, and then different volumes of distilled water were added to the original CH_3CN solution of the **L**/Hg^{2+} complex and the corresponding emissions were monitored.

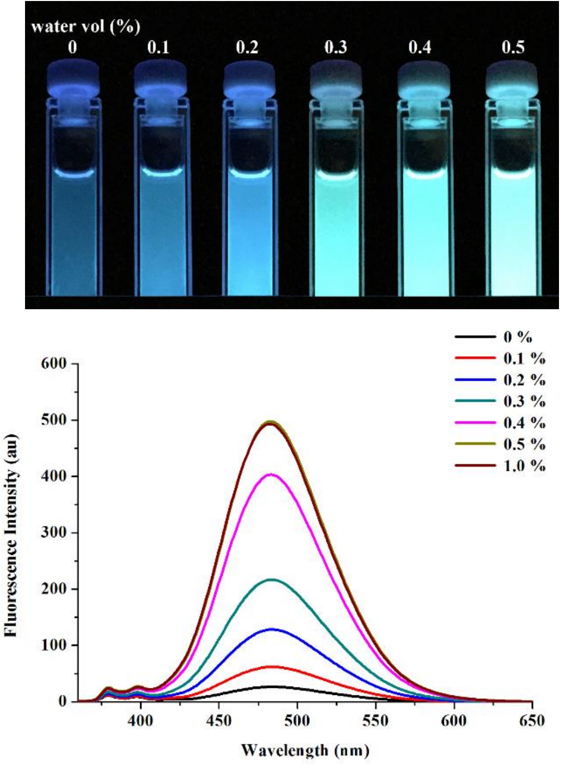

Fig. 4 Fluorescence emission spectra for the **L**/Hg^{2+} complex in CH_3CN with the incremental addition of water and the digital photos of **L**/Hg^{2+} complex in H_2O–CH_3CN mixtures under irradiation at 365 nm.

Fig. 4 illustrates the fluorescence spectral changes of the **L**/Hg^{2+} complex in CH_3CN upon addition of water. Interestingly, the incremental addition of water to the solution of **L**/Hg^{2+} complex resulted in a rapid enhancement of the excimer emission. This process is saturated when about 0.5 % content of water added, with the fluorescence intensity almost revived to original chemosensor **L**. The revival of fluorescence emission is even clearly observed under illumination at 365 nm. Additionally, in order to determine the impact of time on this process, an estimation of the time-dependent fluorescence spectral changes of **L**/Hg^{2+} complex was carried out. As shown in Fig. S6, the fluorescence emission was enhanced immediately after adding water, and the fluorescence intensity could remain stable for a long time. Thus, there must be a strong interaction between the water molecules and **L**/Hg^{2+} complex, which led to the revival of the fluorescence intensity.

For in-depth understanding of the complexation properties of **L** with Hg^{2+} ions in the presence of water molecules, ^1H NMR titration experiments were performed in $CDCl_3/CD_3CN$ (v/v, 2:1) co-solvent. The spectral differences are depicted in Fig. 5. Some significant spectral changes are observed in the ^1H NMR spectra on addition of Hg^{2+}. For example, upon interaction with Hg^{2+}, the proton on the triazole rings undergoes a down-field shift by $\Delta\delta$ 0.26 ppm to 8.07 (red colour), whereas the triazole-*CH₂*-pyrene linker proton is shifted from 5.99 to 5.87 ppm (brown colour). These chemical shift changes reflect the triazole rings in the appendage are involved in the metal ion binding.[13] Furthermore, the chemical shift of aromatic (blue colour) and Ar$CH_2(ax)$O bridge methylene (pink colour) also experienced an up-field shift, which can be attributed to the conformational change of oxacalix[3]arene scaffold upon complexation with the Hg^{2+}. As a matter of fact, it is believed that the conformation of hexahomotrioxacalix[3]arene can be pre-organized for the binding of Hg^{2+} ion in solution in a manner that is similar to the examples described by Shinkai and coworkers.[14] Importantly, it should be noted that the proton signals of pyrene (green colour) obviously shifted to the up-field as the concentration of the Hg^{2+} increased. It suggests that the pyrene moieties still maintain the π–π stacking and exhibit the excimer emission. And this is different from our recent reports, in which the pyrene protons experienced a down-field shift, behaving as the separate of the pyrene moieties with an enhancement of the monomer emission.[8,15]

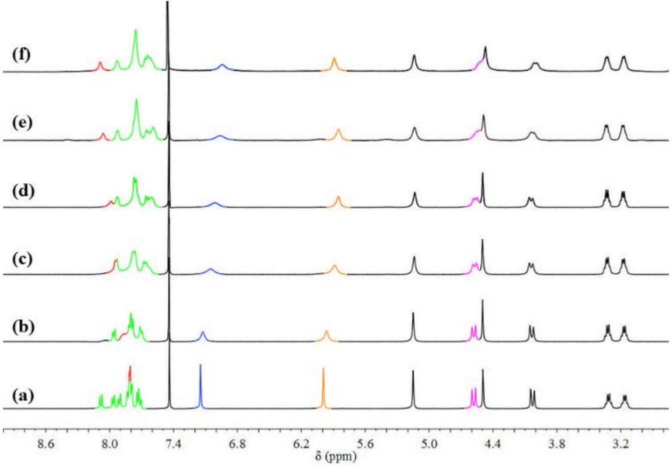

Fig. 5 Partial ^1H NMR spectra of **L** (5.0 mM) in $CDCl_3/CD_3CN$ (v/v, 2:1): (a) **L** only; (b, c, d and e) in the presence of 0.3, 0.5, 0.8 and 1.0 equiv. of Hg^{2+}, respectively; (f) **L**/Hg^{2+} complex in the presence 10 μL D_2O. (full spectra was available in ESI†, Fig. S7).

Further addition of 10 μL D_2O into the solution of **L**/Hg^{2+} complex, no significant chemical shift changes of the protons on the **L**/Hg^{2+} complex were observed (Fig. 5f). This result suggested that the enhancement of the excimer emission of pyrene in the present system should be ascribed to the additional coordination of water molecule to the Hg^{2+} ion rather than the dissociation of the **L**/Hg^{2+} complex and the release of free **L**. Thus we presumed that binding of water molecule to the Hg^{2+} center of the **L**/Hg^{2+} complex causes a redistribution in the energy levels of the **L**/Hg^{2+} complex,[16] or alternatively a water molecule acts as a donor ligand and reduces the electron withdrawing ability of the positive center of the Hg^{2+} ion *versus* the pyrene fluorophore.[6c,17] Both cases thus result in the enhancement of the fluorescence emission of pyrene in the **L**/Hg^{2+} complex.

Additionally, a monomeric analogue **M** was also synthesized as a reference compound and the fluorescence properties were evaluated under the same analytical conditions as used for **L**. It was found that the monomer emission of **M** was also quenched by the addition of 50 equiv. of Hg^{2+}, but the emission intensity cannot be retrieved in the presence of water (Fig. S8, ESI†). These results suggested that the three triazole ligands of the C_3-symmetric homooxacalix[3]arene scaffold play an important role in the complexation between the water molecule and **L**/Hg^{2+} complex.

On the basis of the fluorescence and NMR spectral evidence as well as our previous results, a plausible coordination-assisted fluorescence enhancement detection of Hg^{2+} is therefore depicted in Fig. 6. A usual tetrahedral Hg^{2+}-ligand coordination structure may involve an intramolecular assembly process,[18] and the heavy atom effect of Hg^{2+} can be blocked by the complexation of the water molecule. Thus, the fluorescence emission of pyrene in the **L**/Hg^{2+} complex can be successfully revived.

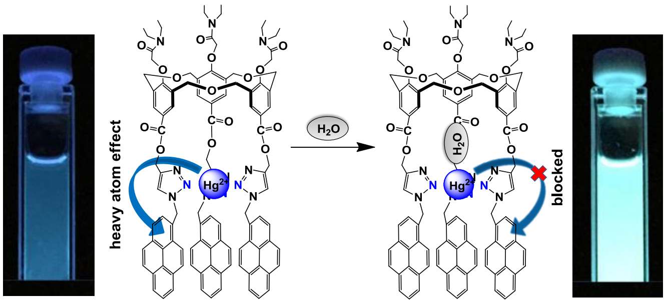

Fig. 6 A plausible mechanism for the heavy atom effect blocked by water in the **L**/Hg^{2+} complex.

In conclusion, the present studies demonstrated a novel fluorescence "on–off–on" chemosensor for Hg^{2+} via a water-assistant blocking heavy atom effect. The information obtained in this work can be considered an interesting contribution to understand the blocking heavy atom effect and could be particularly useful in the design of new fluorescence enhancement chemosensors for HTM ions. Specifically, chemosensor **L** can be utilized as another fluorescent sensor for the detection of water content, and further studies are in progress.

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