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Direct evidence of a blocking heavy atom effect on the water-assisted fluorescence enhancement detection of Hg^{2+} based on a ratiometric chemosensor

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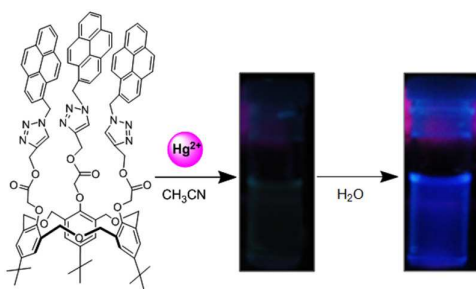
ARTICLE

Direct evidence of a blocking heavy atom effect on the water-assisted fluorescence enhancement detection of Hg^{2+} based on a ratiometric chemosensor

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At the current stage of chemosensor chemistry, the critical question now is whether the heavy atom effect caused by HTM ions can be blocked or avoided. In the present work, we provide unequivocal evidence to confirm that the heavy atom effect of Hg^{2+} is inhibited by water and other solvent molecules based on results using the chemosensor **L**. Most importantly, the heavy atom effect and blocking thereof were monitored within the same system by use of ratiometric fluorescent signal changes of the pyrene motif. These observations not only serve as the foundation for the design of new 'turn-on' chemosensors for HTM ions, but also open up new opportunities for the monitoring of organic reactions.

Introduction

Real-time and real-space analysis of heavy and transition metal (HTM) ions employing fluorescent sensor molecules has received much attention over the past few years.[1] Many metal cations possess a heavy atom effect that usually quenches the fluorescence of organic dye molecules via enhanced spin-orbit coupling,[2] or energy or electron transfer,[3] and the quenching is not only disadvantageous for high signal output upon complexation, but also hampers temporal separation of spectrally similar complexes using time-resolved fluorometry.[4] Therefore, much research has lately been devoted to designing fluorescent probes that show complexation-induced fluorescence enhancement based on sensing mechanisms including photoinduced electron transfer (PET),[5] photoinduced charge transfer (PCT),[6] formation of monomer/excimer,[7] and fluorescence resonance energy transfer (FRET).[8] However, in the field of sensor research, the photophysical and photochemical mechanisms of the heavy atom effect are rather diverse and few attempts have been made to establish general concepts for rational probe design.[9] This is despite it been many decades since McClure (1952)[2] and Wehry (1972)[3] demonstrated the principle of heavy atom effect quenching on fluorescence emission. This is in-part due to literature reports focusing on the "Why" of the intrinsic quenching of HTM ions, but not provided the "How" to block the quenching effect. Therefore, the question now is whether the heavy atom effect caused by HTM ions can be blocked or indeed avoided? Undoubtedly, understanding this critical question is very important for future HTM probe design.

When compared to the majority of reported fluorescent sensors which display an increase or decrease in the emission intensity upon binding to the target species, the use of ratiometric fluorescent sensors, which use the ratio of two fluorescent bands instead of the absolute intensity of one band, and for which the measuring process

of the analyte is more accurate and sensitive, has seen increased interest of late.[10] For example, pyrene is one of the most useful tools when constructing ratiometric sensors due to its novel signal excimer-to-monomer emission (IE/IM), and is very sensitive to conformational change.[7] Additionally, in order to clarify the question regarding the heavy atom effect caused by HTM ions, two events, namely 1) the process of quenching emission by heavy atom effect; and 2) the process of inhibiting the heavy atom effect; need to be distinguished by different fluorescent signals. As a consequence, a ratiometric fluorescent sensor is ideally suited to address the issue. To the best of our knowledge, few studies have been paid to such questions thus far.[9d,e] In particular, there are no reports on the fluorescence monitoring of the procedure of the heavy atom effect and the blocking thereof within the same system.

Results and discussion

Previously, we have reported that the pyrene-linked homooxacalix[3]arene-derived chemosensor **L** (Fig. 1a) exhibited high selectivity for the Pb^{2+} ion by use of a switchable ratiometric detection signal output of the excimer and monomer emission of pyrene in both organic and organic/aqueous media.[11] However, the fluorescence quenching by the Pb^{2+} ion could be observed if a fluorophore with two triazole moieties incorporated onto a tailored calix[4]arene scaffold was employed.[12] The different fluorescence changes can be attributed to the three nitrogen triazole ligands of **L**; the ionophore is capable of providing an adequate electronic effect to isolate the Pb^{2+} ion from the fluorophore and thereby maintaining the monomer emission of pyrene.[11] Interestingly, further experiments indicated that **L** exhibited an impressive quenching of both excimer and monomer signal outputs of pyrene in the presence of small amounts of Cu^{2+} and Hg^{2+} in pure organic solvents (Fig. 1b), but the monomer emission of pyrene appeared slightly enhanced during the

addition of Cu^{2+} and Hg^{2+} to organic/aqueous solutions of **L** (Fig. S1-S5). We assumed that the water molecules in this system were participating in the sensing procedure of **L** with Hg^{2+} and that the heavy atom effect of the HTM ions may be directly blocked in some manner. Hence, given this work, we report here the first evidence of the blocking of the heavy atom effect via the water-assisted fluorescence enhancement detection of Hg^{2+} by utilizing the unique fluorescent ratiometric signal of the excimer/monomer emission (IE/IM) of the pyrene moiety appended to a C_3-symmetric homooxacalix[3]arene scaffold.
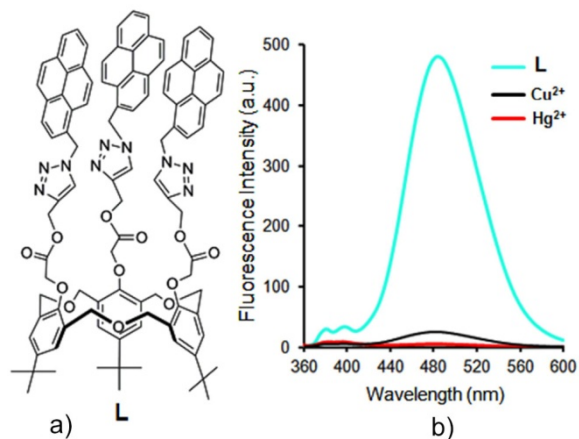

Fig. 1 (a) Chemical structure of **L**, (b) fluorescence spectral changes of **L** (1.0 μM) upon addition of Cu^{2+} and Hg^{2+} ions as their perchlorates in CH_3CN solution at 298 K (λ_{ex} = 343 nm).

Fig. 1b illustrates the fluorescence spectral changes of the chemosensor **L** upon addition of Cu^{2+} and Hg^{2+} ions in pure acetonitrile solution, and it is evident that both the excimer and monomer emission of pyrene have been dramatically quenched. The pyrene moiety was appended to the calix[3]arene scaffold by triazole groups to form a strong excimer in the free state, and this results in a characteristic decrease of the excimer emission intensity and a concomitant increase of monomer emission intensity when the triazole rings selectively bind to the analytes.[11] Consequently, the excimer emission of the pyrene when quenched by the Cu^{2+} and Hg^{2+} ions can be attributed to the coordination induced structural changes of **L**, and the monomer emission quenching is generally ascribed to the heavy atom effect of Cu^{2+} and Hg^{2+}.

As mentioned previously, we hypothesized that the water molecules may be participating in the complexation of **L** with Hg^{2+}. Thus, in order to systematically investigate the role of the water molecules in the **L**-Hg^{2+} complex, the fluorescence spectral properties of chemosensor **L** were studied using different concentrations of $Hg(ClO_4)_2$, water and binding time. Fig. 2a shows the fluorescence spectral changes of **L** upon addition of different concentrations of Hg^{2+} (1, 10, 50, 100 μM) ions, respectively, in acetonitrile solution. It was found that both the excimer and monomer emission intensities of pyrene decreased, even after 48 h (Fig. 3a). This result strongly suggested that the heavy atom effect of Hg^{2+} occurred in pure CH_3CN solution.
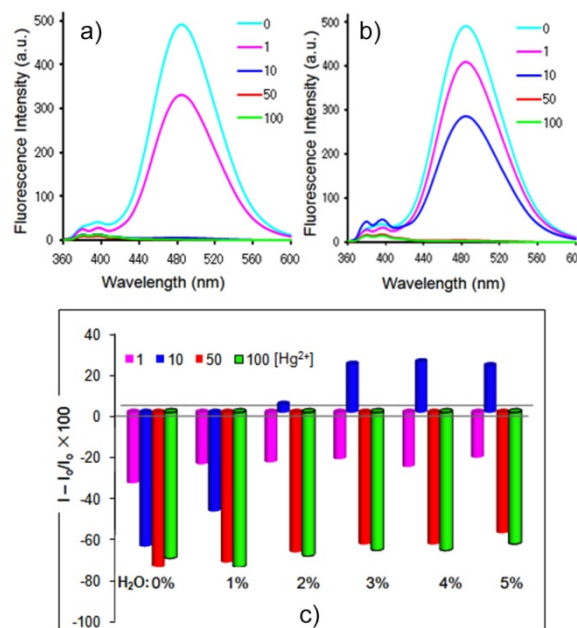

Fig. 2 Fluorescence spectral changes of **L** (1.0 μM) upon addition of different concentrations of $Hg(ClO_4)_2$ (1, 10, 50, 100 μM) and determined immediately at 298 K in a) CH_3CN solution, b) H_2O/CH_3CN (v/v, 5:95), c) fluorescence intensity changes (($I-I_o$)/$I_o \times 100$ %) of **L** in different concentrations of $Hg(ClO_4)_2$ and water. I_o is the monomer emission intensity of **L** at 396 nm, and I is the fluorescent intensity after adding Hg^{2+} ions (λ_{ex} = 343 nm).
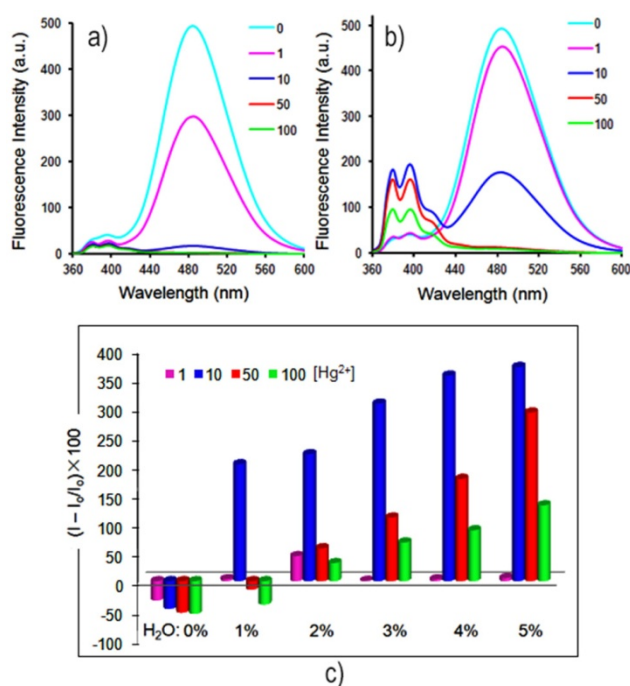

Fig. 3 Fluorescence spectral changes of **L** (1.0 μM) upon addition of different concentrations of $Hg(ClO_4)_2$ (1, 10, 50, 100 μM) and determined after 48 h at 298 K in a) CH_3CN solution, b) H_2O/CH_3CN (v/v, 5:95), c) fluorescence intensity changes (($I-I_o$)/$I_o \times 100$ %) of **L** in different concentrations of $Hg(ClO_4)_2$ and water. I_o is the monomer emission intensity of **L** at 396 nm, and I is the fluorescent intensity after adding Hg^{2+} ions (λ_{ex} = 343 nm).

However, if different volumes of distilled water (H_2O/CH_3CN (v/v): 1 %, 2 %, 3 %, 4 %, 5 %) were added to the original acetonitrile solution of the **L**-Hg^{2+} complex, and the fluorescence determined immediately (Fig. S6), it was found that the excimer emission of pyrene was quenched, though the intensity of the monomer emission of the pyrene appeared slightly enhanced in the case of 10 equiv. of Hg^{2+} ions in **L** solution if the % volume of water was over 1 % (Fig. 2b). By contrast, the monomer emission of pyrene was still quenched by larger concentrations of Hg^{2+} ions (50 and 100 equiv.), no matter what the ratio of water (Fig. 2c) employed. Interestingly, if the fluorescence spectral of such species were determined again after 48 h, it was noted that nearly all of the monomer emission of pyrene of the **L**-Hg^{2+} complex has been retrieved (Fig.3b and Fig. S7), and the fluorescence intensity of the monomer emission of pyrene discernibly increased with a dependence on the different concentrations of Hg^{2+} ions and water volume as shown in Fig. 3c. In particular, in the case of 10 equiv. of Hg^{2+} ions, it was evident that the monomer emission intensity of pyrene increased from 200 % to 400 % on increasing the water content from 1 % to 5 % compared to the original monomer emission intensity of pyrene on free **L** (Fig. 3c). Therefore, based on the above fluorescence spectral changes, we concluded that the water molecules herein may be participating in the complexation procedure of **L** with Hg^{2+}, and the unequivocal enhancement of the monomer emission of pyrene moiety can be attributed to the water co-coordination induced inhibition of the heavy atom effect of Hg^{2+}.
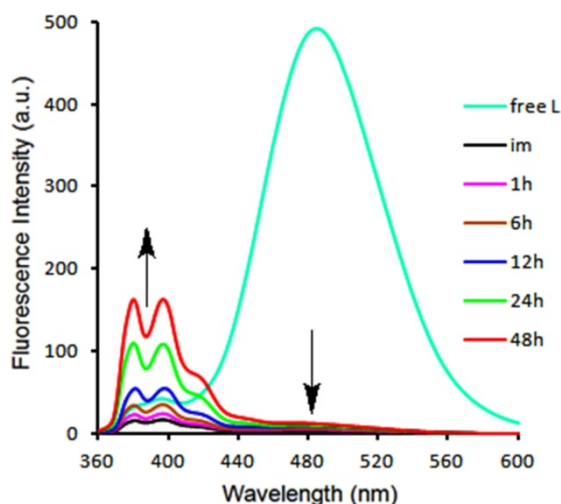

Fig. 4 Fluorescence spectral changes of **L** (1.0 μM) upon addition of 50 equiv. of Hg^{2+} ions in H_2O/CH_3CN (v/v, 5:95) solution and determined over different times at 298 K (λ_{ex} = 343 nm).

In an effort to gain detailed information on the monomer emission enhancement binding mechanism of **L**-Hg^{2+} by water molecules, an estimation of the time-dependent fluorescence spectral changes of **L**-Hg^{2+} was carried out in the presence of 5 % water volume (H_2O /CH_3CN , v/v, 5:95). As shown in Fig. 4, the monomer and excimer emissions of free **L** appeared at the typical emission wavelengths of 396 and 485 nm, respectively, whilst the determined fluorescence spectral

changes indicated that both emissions, in particular of the excimer emission, was completely quenched by the addition of 50 equiv. of Hg^{2+}. However, on ageing of the original sample, monitoring of the fluorescence spectral demonstrated that the maximum monomer emission at 396 nm clearly increased, whilst the excimer emission remained completely quenched. The increase in the fluorescence was supported by the quantum yield (ϕ_f),[13] which increased from 0.019 to 0.137. This result clearly revealed that i) the water molecules were coordinated to the Hg^{2+} ion rather than being removed from the **L**-Hg^{2+} complex; ii) the formation of the binary **L**-Hg^{2+} complex is very fast, whilst the formation of the ternary **L**-Hg^{2+}-H_2O complex is slow. Therefore, the events associated with the heavy atom effect and the inhibition thereof can be accurately distinguished within the same system by different fluorescent signals; iii) the heavy atom effect of Hg^{2+} was reasonable proved to be blocked by the co-ordination of water molecule. Moreover, Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF) was also used to investigate the binding. When Hg^{2+} ion was added to a CH_3CN solution of **L** in the presence of 5 % water, a new peak appeared at m/z 1894.785, corresponding to [**L**+ Hg^{2+} + 3H_2O + H]$^+$(Fig. S8).
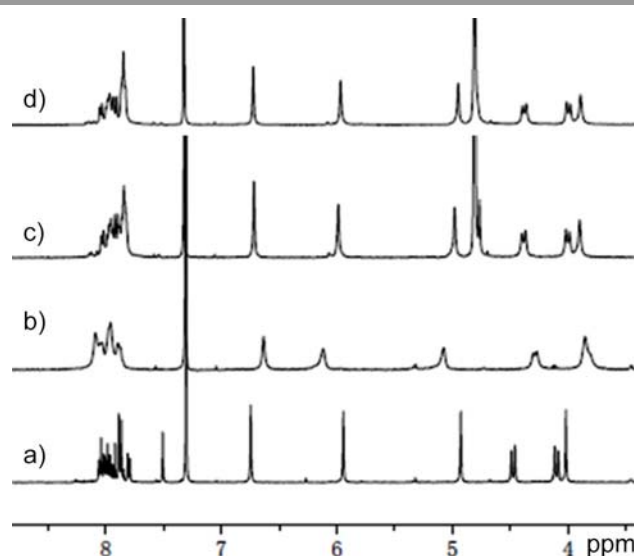
Fig. 5 Partial ^{1}H NMR spectral of **L** (3.0 mM) in $CDCl_3/CD_3CN$ (10:1, v/v): a) free **L**, b) **L**-Hg^{2+} complex; and **L**-Hg^{2+} complex in the presence 30 μL D_2O c) determined immediately, d) determined after 48 h at 298 K.

In order to obtain more detailed information on the complexation properties of **L** with Hg^{2+} ions in the presence of water molecules, ^{1}H NMR titration experiments were performed in a mixture of $CDCl_3/CD_3CN$ (10:1, v/v). The spectral changes are shown in Fig. 5. Upon addition of 1.0 equiv of Hg^{2+} ions to the solution of **L**, as expected, the chemical shift of the triazole ring proton at δ 7.54 (Fig. 5a) exhibited a significant downfield shift and was now buried inside the peaks of the pyrene protons (Fig. 5b). The OCH_2-triazole linker proton and the proton proximal to the pyrene were also shifted downfield, which suggested that the three triazole moieties of **L** were participating in the complexation with Hg^{2+}. Furthermore,

we also noted that the Ar*CH₂*O-bridge linker protons $H_{g(ax)}$ and $H_{g(eq)}$ and the aromatic protons were shifted upfield, which can be attributed to the conformation changes of **L** upon complexation with Hg^{2+} ion. On addition of 30 μL D_2O to the solution of **L**-Hg^{2+} complex, no further significant chemical shift changes of the protons on the complex of **L**-Hg^{2+} were observed over different binding times (Fig. 5c and 5d). This result suggested that the enhancing of the monomer emission of pyrene in the present system should be ascribed to the additional coordination of water molecules to the Hg^{2+} ion rather than Hg(II)-catalysed hydrolysis of the ester groups of the calix[3]arene ligand employed. When water molecules were added to a solution of the **L**-Hg^{2+} complex, it is presumed that binding of water to the Hg^{2+} center of the **L**-Hg^{2+} complex causes a redistribution in the energy levels of the **L**-Hg^{2+} complex,[14] or alternatively a water molecule acts as a donor ligand and reduced the electron withdrawing ability of the positive center of Hg^{2+} ion *versus* the pyrene fluorophore.[15] Both cases thus lead to an enhancement of the monomer emission of pyrene in the **L**-Hg^{2+} complex. Following this observation, we found that other solvents such as CH_3OH and CH_3CH_2OH also have the ability to revive the monomer emission of the **L**-Hg^{2+} complex (Fig. S9).
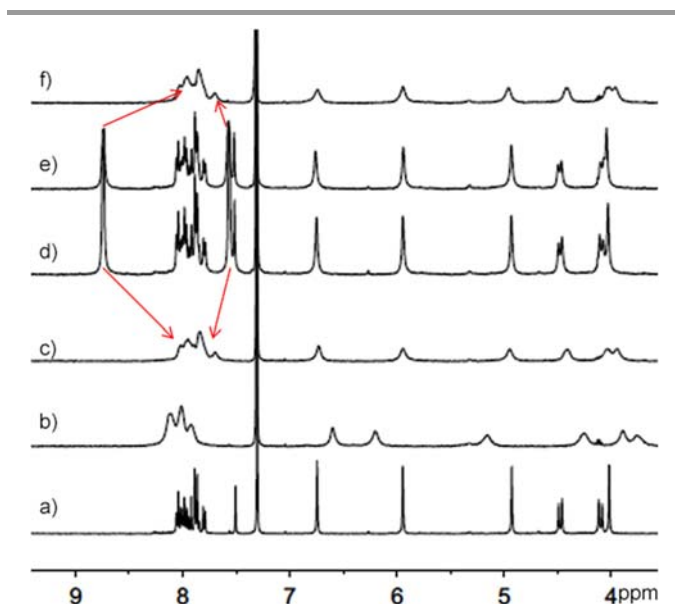

Fig. 6 Partial ^1H NMR spectra of **L** (3.0 mM) in $CDCl_3/CD_3CN$ (10:1, v/v): a) free **L**, b) **L**-Hg^{2+} complex (1:1), c) **L**-Hg^{2+} complex (1:1) in the presence 1.0 equiv of 4,4'-bipyridine, d) **L** with 4,4'-bipyridine (1:1), e) **L** with 4,4'-bipyridine in the presence of 0.5 equiv of $Hg(ClO_4)_2$, f) **L** with 4,4'-bipyridine in the presence of 1.0 equiv of $Hg(ClO_4)_2$ at 298 K.

More interestingly, when 1.0 equiv of 4,4'-bipyridine was added to the solution of **L**-Hg^{2+} complex, as shown in Fig. 6, it can be see that an obvious chemical shift change for the protons on the 4,4'-bipyridine was observed in the ^1H NMR titration spectra. This may be attributed to coordination behaviour of the Hg^{2+} ion, which binds to the lone-pair of electrons on the pyridine nitrogen atom of the guest, together with the inclusion ability of the hydrophobic **L**. This result indicated that 4,4'-bipyridine could also serve as a ligand to the Hg^{2+} centre via the hydrophobic cavity of the **L**-Hg^{2+} complex. Considering that metal-coordination based three-dimensional

hydrophobic cavities of calix[*n*]arenes have good selectivity for neutral molecules,[16] it is expect that the present study will have potential applications in the development of new types of fluorescent chemosensor for the analysis organic reactions.[17] For example, the complexation of **L**-Hg^{2+} can be exploited to monitor the reaction procedure of 4,4'-bipyridine by fluorescence thereby determining its consumption (Fig. S10-S13).

Additionally, we assumed that the three triazole ligands of the C_3-symmetric scaffold of the homooxacalix[3]arene can play an important role in activating Hg^{2+} to adopt the tetrahedral coordination mode. To further confirm this assumption, the monomeric compound **M** (Fig. S14) was 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 5 % volume ratios of water and over prolonged binding times (Fig. S15-S16). It should also be noted that similar revive monomer emission of pyrene can be detected for the **L**-Cu^{2+} complex in the presence of water, CH_3OH, CH_3CH_2OH and 4,4'-bipyridine (Fig. S17). Thus, based on the above observations, the plausible coordination-assisted fluorescence enhancement detection of HTM ions by inhibiting the heavy atom effect is shown in Fig. 7.
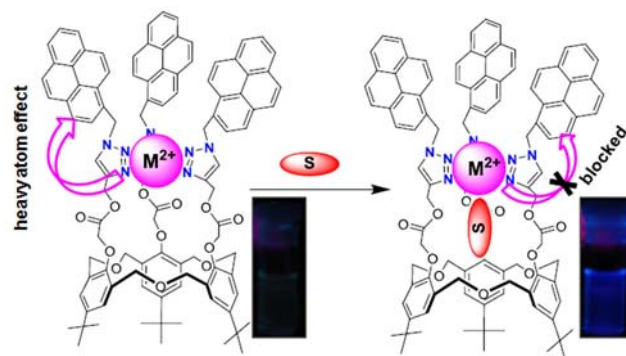

Fig. 7. A plausible mechanism for the heavy atom effect blocked by solvent in the **L**-M^{2+} complex (M= Hg, Cu, and S= H_2O, CH_3OH, CH_3CH_2OH).

Conclusions

In summary, the present studies provide unequivocal evidence that the heavy atom effect of Hg^{2+} can be blocked by water molecules when using the pyrene linked triazole modified homooxacalix[3]arene chemosensor **L**. Furthermore, the processes of the heavy atom effect and inhibition thereof can be clearly distinguished in the same system by use of simple ratiometric fluorescent signal changes of pyrene. To the best of our knowledge, such accurate fluorescent signal monitoring of the activity of the heavy atom effect in sensor chemistry using a ratiometric probe has not previously been demonstrated. The observations herein provide the foundations for the design of new 'turn-on' chemosensors for HTM ions, and opens up a new opportunity for the monitoring of organic reactions.

Notes and references

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† Footnotes should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectra data, and crystallographic data.

Electronic Supplementary Information (ESI) available: details of analysis data including UV-vis and fluorescence spectral or other electronic format see DOI: 10.1039/c000000x/

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