

Direct evidence of a blocking heavy atom effect on the water-assisted fluorescence enhancement detection of Hg²⁺ based on a homooxalix[3]arene derived ratiometric chemosensor

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Abstract: 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 Hg²⁺ is inhibited by water 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.

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 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 in the same system.

Previously, we have reported that the pyrene-linked homooxalix[3]arene-derived chemosensor **L** (Figure 1a) exhibited high selectivity for the Pb²⁺ ion by use of a switchable ratiometric detection signal output of the excimer and monomer emission of pyrene in both organic and organic/aqueous solution.¹¹ However, we also noted that **L** exhibited an impressive quenching of both excimer and monomer signal outputs of pyrene for small amounts of Cu²⁺ and Hg²⁺ in pure organic solvents (Figure 1b), but the monomer emission of pyrene appeared slightly enhanced during the addition of Cu²⁺ and Hg²⁺ to the organic/aqueous solution of **L** (Figures S1-S2). We assumed that the water molecules in this system were participating in the sensing procedure of **L** with Hg²⁺ and heavy atom effect of 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 water-assisted fluorescence enhancement detection of Hg²⁺ by utilizing the unique fluorescent ratiometric signal of the excimer/monomer emission (IE/IM) of the pyrene moiety appended to a C₃-symmetric homooxalix[3]arene scaffold.

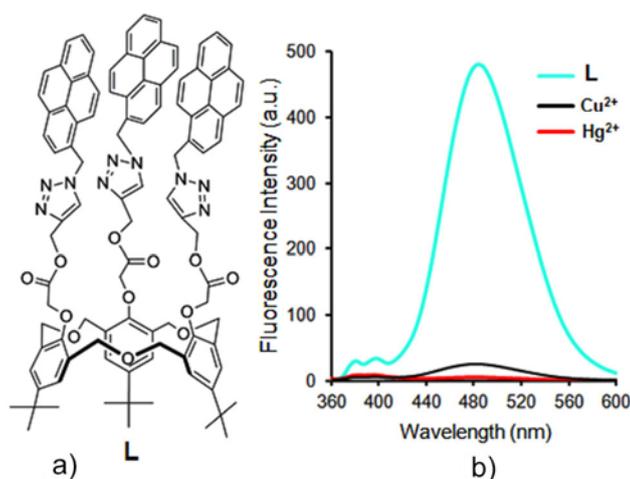


Figure 1. (a) Chemical structure of **L**, (b) fluorescence spectral changes of **L** (1.0 μ M) upon addition of Cu²⁺ and Hg²⁺ ions as their perchlorates in CH₃CN solution at 298 K ($\lambda_{\text{ex}} = 343$ nm).

Figure 1b illustrates the fluorescence spectra changes of the chemosensor **L** upon addition of Cu²⁺ and Hg²⁺ 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 Cu²⁺ and

Hg^{2+} ions can be attributed to the coordination induced structure 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 $\text{Hg}(\text{ClO}_4)_2$, water and binding time. Figure 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 (Figure 3a). This result strongly suggested that the heavy atom effect of Hg^{2+} occurred in the pure CH_3CN solution. However, if different volumes of distilled water ($\text{H}_2\text{O}/\text{CH}_3\text{CN}$ (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 (Figure S3), it was found that the excimer emission of pyrene was quen-

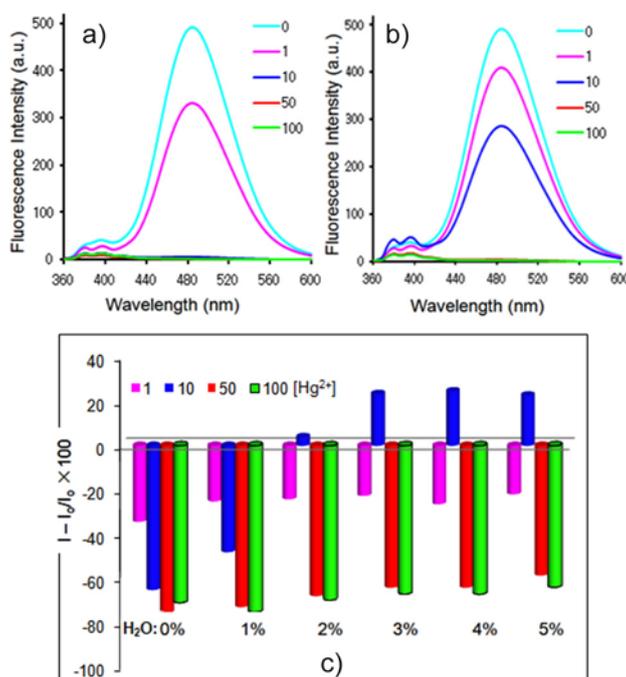


Figure 2. Fluorescence spectral changes of **L** (1.0 μM) upon addition of different concentrations of $\text{Hg}(\text{ClO}_4)_2$ (1, 10, 50, 100 μM) and determined immediately at 298 K in a) CH_3CN solution, b) $\text{H}_2\text{O}/\text{CH}_3\text{CN}$ (v/v, 5:95), c) fluorescence intensity changes $((I - I_0)/I_0 \times 100\%)$ of **L** in different concentration of $\text{Hg}(\text{ClO}_4)_2$ and water. I_0 is monomer emission intensity of **L** at 396 nm, and I is the fluorescent intensity after adding Hg^{2+} ions ($\lambda_{\text{ex}} = 343 \text{ nm}$).

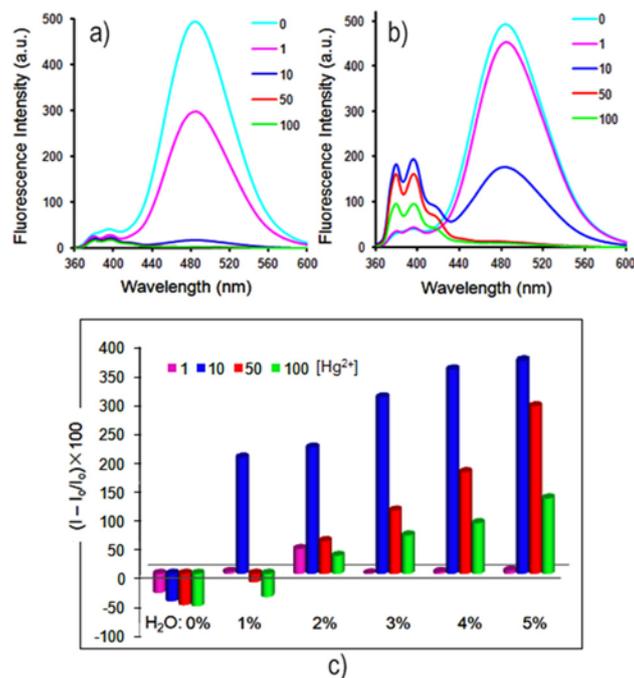


Figure 3. Fluorescence spectral changes of **L** ($1.0 \mu\text{M}$) upon addition of different concentrations of $\text{Hg}(\text{ClO}_4)_2$ (1, 10, 50, 100 μM) and determined after 48 h at 298 K in a) CH_3CN solution, b) $\text{H}_2\text{O}/\text{CH}_3\text{CN}$ (v/v, 5:95), c) fluorescence intensity changes $((I-I_0)/I_0 \times 100 \%)$ of **L** in different concentration of $\text{Hg}(\text{ClO}_4)_2$ and water. I_0 is monomer emission intensity of **L** at 396 nm, and I is the fluorescent intensity after adding Hg^{2+} ions ($\lambda_{\text{ex}} = 343 \text{ nm}$).

ched, 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 % (Figure 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 (Figure 2c) employed. Interestingly, if the fluorescence spectra 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 (Figure 3b and Figure S4), 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 Figure 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** (Figure 3c). Therefore, based on the above fluorescence spectral changes, we concluded that the water molecules herein must 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-ordination induced inhibition of the heavy atom effect of Hg^{2+} .

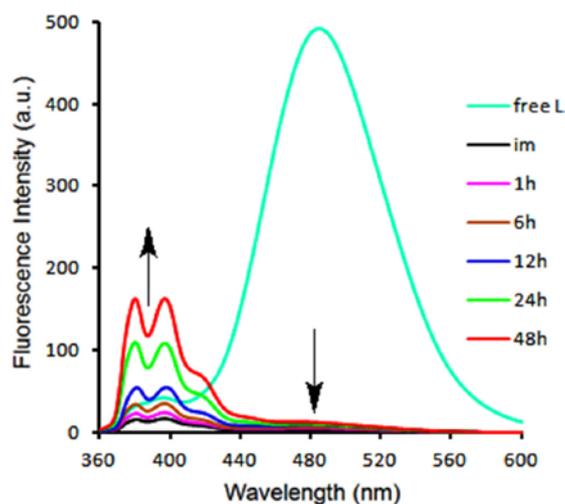


Figure 4. Fluorescence spectral changes of **L** ($1.0 \mu\text{M}$) upon addition of 50 equiv. of Hg^{2+} ions in $\text{H}_2\text{O}/\text{CH}_3\text{CN}$ (v/v, 5:95) solution and determined over different times at 298 K ($\lambda_{\text{ex}} = 343 \text{ nm}$).

In an effort to gain more 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 ($\text{H}_2\text{O}/\text{CH}_3\text{CN}$, v/v, 5:95). As shown in **Figure 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 spectra demonstrated that the maximum monomer emission at 396 nm was **clearly** increasing, whilst the excimer emission remained completely –**do you mean unchanged?**. This result clearly revealed that i) **water molecules as ligand coordination to the Hg^{2+} ion rather than remove it from L-Hg^{2+} complex. Otherwise, the retrieve excimer emission of pyrene on sensor **L** should be observed** – sorry, this still makes no sense (also see **your answer**); ii) the formation of the binary L-Hg^{2+} complex is very fast, whilst the formation of the ternary $\text{L-Hg}^{2+}\text{-H}_2\text{O}$ 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-coordination of water molecule. 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 redistribution in the energy levels of the L-Hg^{2+} complex. For example, the degenerate orbitals of the Hg^{2+} ions (6p) are stabilized and thus decrease the spin-orbit coupling ability of the Hg^{2+} to the pyrene moieties,¹² or alternatively a water molecule can act as a sacrificial donor and reduce the electron withdrawing ability of the positive center of Hg^{2+} ion **versus** the pyrene fluorophore.¹³ Both cases can lead to **the retrieve??** and enhancement of the monomer emission of pyrene in the L-Hg^{2+} complex. Considering the $5d^{10}6s^2$ electronic configuration of mercury, two-coordinate linear and four coordinate tetrahedral species are commonly observed for Hg^{2+} .¹⁴ Accordingly, 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** (Figure S5) 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 prolonged binding times (Figures S6-S7). Thus, based on the above observations, plausible water coordination-assisted fluorescence enhancement detection of Hg^{2+} by inhibiting the heavy atom effect is shown in Figure 5. Furthermore, considering that metal-coordination based three-dimensional hydrophobic cavities of calix[*n*]arenes have good selectivity for neutral molecules,¹⁵ it is expected that the present study will have potential applications in the development of new types of fluorescent chemosensor for the analysis organic reactions.¹⁶ For example, the complexation of **L**- Hg^{2+} can be exploited to monitor the interaction with 4,4'-bipyridine via the fluorescence spectra of the **L**- Hg^{2+} complex (Figures S8-S10).

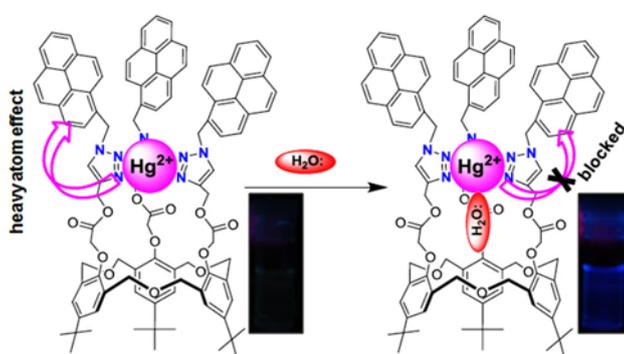


Figure 5. A plausible mechanism for the heavy atom effect blocked by water in the **L**- Hg^{2+} complex.

In conclusion, 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. More experimental studies on this topic are currently ongoing.

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