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A dioxadithiaazacrown ether-BODIPY dyad Hg^{2+} complex for detection of L-cysteine: Fluorescence switching and application on soft material

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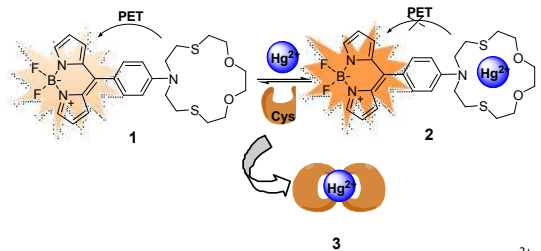
Navdeep Kaur, Paramjit Kaur* and Kamaljit Singh*

A Hg^{2+} coordinate complex of a 1,4-dioxa-7,13-dithia-10-azacyclopentadecane-BODIPY dyad 1 recognises L-cysteine (cys), selectively, over other amino acids via reversible complexation/decomplexation and show switching of fluorescence upon sequential addition of Hg^{2+} and cys solutions. Further, addition of the dyad to the Hg^{2+} cholate hydrogel is attended by gel to sol phase transition.

The mercapto biomolecules play a crucial role in important chemical and physiological processes in organisms.[1] Among these, L-cysteine (cys) plays crucial role in plasma. The abnormal levels of cys result in many disorders linked to the liver damage, slow growth in children, skin lesions etc.[1a,2] Consequently, for prevention of such diseases, many research groups have focussed their attention on the development of probes for the detection of mercapto biomolecules.[3] Over the traditional methods, like gas chromatography,[4] electrochemical methods,[5] mass spectrometry,[6] the fluorescence probes are widely being used to recognise the mercapto biomolecules because of the simplicity, high sensitivity and selectivity.[3] However, among these, the reversible probes may have edge over the irreversible counterparts as the possible retention of the latter in the cells could cause damage to the cells, thus affecting the further studies.[7] Thus, development of reversible fluorescent probes that could avoid toxic cellular retention for the detection of mercapto biomolecules is highly desired. Most of the sensing protocols for thiols have been executed (i) through covalent interactions between the receptor and the analyte such as Michael addition,[8] cyclisation with aldehyde[9] or cleavage reactions promoted by the thiols.[10] The changes are manifested in terms of the changes in photo-physical properties of the receptor, (ii) non-covalent interaction between the receptor and the analyte (chemosensing ensemble),[11] where the fluorescent indicator is displaced by the analyte, and (iii) using the redox chemistry of thiol, involving absorption studies.[12] Since our research group is involved in development of chemosensors/chemodosimeters for different analytes,[13] in one of our recent works,[13e] a molecular probe **1** (Scheme 1) derived by conjugating the metal ion binding crown ether **1** with the fluorescent signalling handle, BODIPY, for the detection of Pd^{2+} ions, we noticed that the *turn-off* fluorescence of **1** resulting from photoinduced electron transfer (PET) was *turned-on* in the presence of Hg^{2+} ions as a result of restricted PET. The sensing protocol reverses in the presence of cys, evidently as a consequence of sequestering of Hg^{2+} by cys from **1**, as depicted in Scheme 1. To capitalize on this observation, we planned to develop **1**:Hg^{2+} complex **2**, formed *in situ,* as a fluorescent sensing probe, which could exhibit selectivity and sensitivity to mercapto biomolecules via a reversible decomplexation/complexation mechanism. This is one of the examples[14] where such an approach is employed for the detection of a mercapto biomolecule. Additionally, we have explored the possibility of exploiting the changes in the fluorescence of **1** upon addition of Hg^{2+} and/or cys for fabricating INHIBIT logic gate. Further, we also demonstrate application of **1** for selective determination of toxic Hg^{2+} in the soft materials.

The dyad **1** was synthesised following the route already described and was satisfactorily characterised using spectroscopic data.[13e] The fluorescence spectrum of **1** (5×10^{-6} M, in CH_3CN) is characterised by a very weak intensity emission band at 520 nm ($\phi_f = 0.0018$, see †ESI) when excited at 488 nm. The weakly fluorescent nature of **1** has been ascribed to the PET from the crown unit to the BODIPY core.[13e] The electronic absorption spectrum of **1** (1×10^{-5} M, in CH_3CN) exhibits an intense absorption band at 484 nm and a shoulder at 538 nm, attributed to internal charge-transfer (ICT) transition, responsible for the pink color of **1** (Fig. S1, see †ESI). Upon addition of aqueous solution of Hg^{2+} ions (2.87×10^{-7} M to 1.14×10^{-5} M) to a solution of **1** (5×10^{-6} M)



Scheme 1 Reversible fluorescence changes in **1** upon addition of Hg^{2+} and cys.

saturation in the fluorescence intensity was achieved upon adding 1.11 x 10^{-5} M of Hg^{2+} (Fig. S1, see †ESI). While a similar saturation in the absorbance was achieved when Hg^{2+} solution was added to a concentration of 2.22 x 10^{-5} M (Fig. S2, see †ESI). Upon above addition of Hg^{2+}, considerably enhanced intensity of the emission band (ϕ_f = 0.2595, see †ESI) consequent to the interaction of Hg^{2+} with the crown unit receptor of **1**, and a bathochromic shift in the intense absorption band, accompanied by a *naked-eye* color change, were observed. The analysis of the titration data and theoretical calculations were suggestive of a 1:1 stoichiometry of the most stable **1**:Hg^{2+} species **2** with binding constant value $log\ \beta_{1,1}$ = 4.55. Further, **2** showed excellent selectivity for cys over other amino acids such as L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, L-histidine, L-proline, L-leucine, L-isoleucine, L-lysine, L-methionine, L-ornithine, L-phenylalanine, L-proline, L-serine, L-threonine and L-valine (Fig. 1).

On successive addition of an aqueous solution of cys (2.85 x 10^{-7} M to 2.85 x 10^{-6} M, in water) to a solution of **2** (1.64 x 10^{-5} M, in CH_3CN), the intensity of the emission band of **2** at 520 nm decreases regularly and gets stabilised when addition of 2.57 x 10^{-6} M solution is achieved (Fig. 2). This has been attributed to the reversed PET consequent to obvious snatching of Hg^{2+} ions by the cys, as shown in Scheme 1, causing the fluorescence spectra of **2** to match gradually with that of **1** (ϕ_f = 0.0027, see †ESI) releasing the complex **3** in solution with binding constant $log\ \beta$ = 6.33 (For detail see †ESI). Such a reversal event was also observed in the absorption spectrum of **2**, wherein upon gradual addition of a solution of cys (2.85 x 10^{-7} to 4.28 x 10^{-6} M, in H_2O), a hypsochromic shift of the intense absorption band at 498 nm to 484 nm as well as the appearance of the shoulder resulted along with a naked-eye color change from light orange to pink (Fig. 3). This absorbance change reached the saturation point at the addition of 4 x 10^{-6} M solution of cys. The release of **1** from **2** and the formation of **3** resulted from the snatching of Hg^{2+} by cys, was

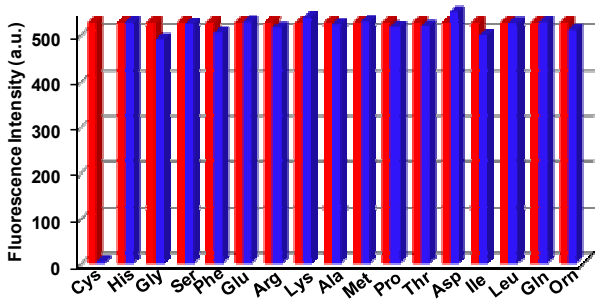


Fig. 1 Changes in the emission spectra of **2** (1.64 x 10^{-5} M, in CH_3CN, red) upon addition of different amino acids (2.85 x 10^{-6} M, in water, blue).

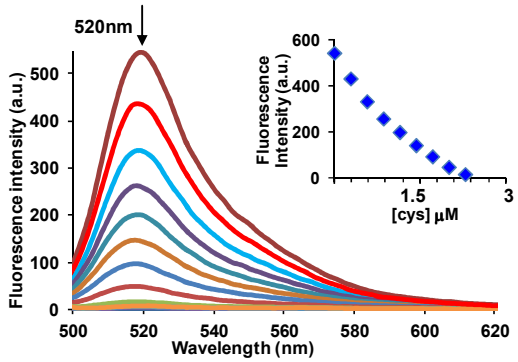


Fig. 2 Changes in the emission spectra of **2** (1.64 x 10^{-5} M, in CH_3CN), on addition of different concentrations of cys (2.85 x 10^{-7} M to 2.85 x 10^{-6} M, in water) in CH_3CN.

further confirmed from the high resolution mass spectrum (HRMS) which shows a peak at *m/z* 443.0, corresponding to $[Hg^{2+}+2cys-H]^+$ (Fig. 4a). From the ^1H NMR spectral data (Fig. 4b, for detail see †ESI) we could notice that the resulting

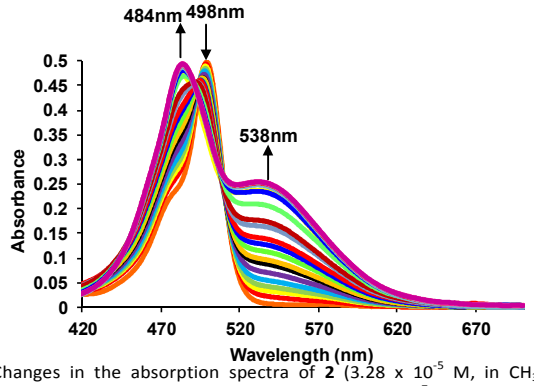


Fig. 3 Changes in the absorption spectra of **2** (3.28 x 10^{-5} M, in CH_3CN) on addition of different concentration of cysteine (2.85 x 10^{-7} to 4.28 x 10^{-6} M, in H_2O), in CH_3CN.

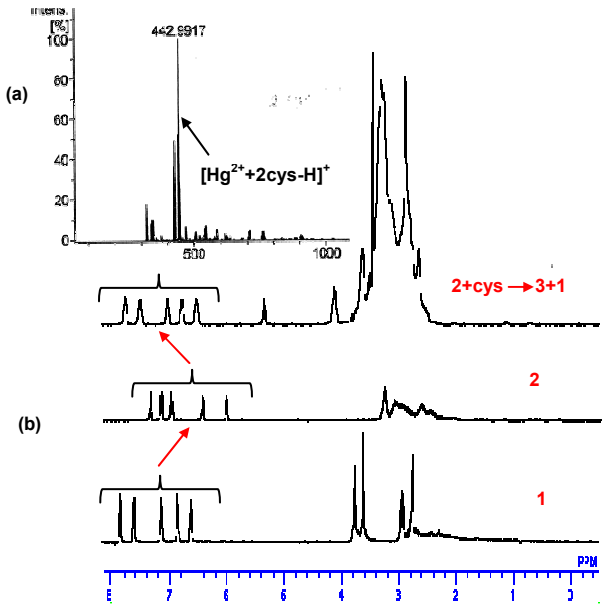


Fig. 4 (a) HRMS spectrum of **2** after addition of cys (b) ^1H NMR spectra of **1**, **2** and **2**+cys showing shift in signal positions.

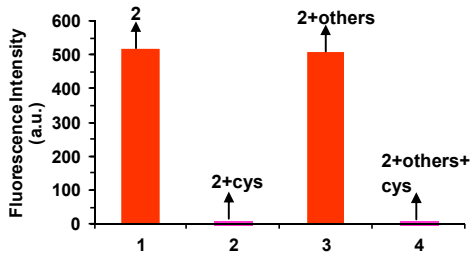


Fig. 5 Changes in the emission intensity (1) of **2** (1.64 x 10^{-5} M, in CH_3CN) upon addition of (2) cys (2.85 x 10^{-6} M, in water) (3) other amino acids (2.85 x 10^{-6} M, in water) (4) cys in the presence of other amino acids

chemical shifts of **1** in the presence of Hg^{2+} return to the original values upon the addition of cys. The calculated detection limit of 1.60 x 10^{-8} M is significantly lower than most of the probes reported in the literature (Table S2, see †ESI). In order to ascertain the thiol selectivity of **2**, and to rule out the interference from the other amino acids without thiol groups, listed above, competitive experiments were performed. No notable change was observed, which is a significant consideration from practical applications point of view (Fig. 5)

Interestingly, the reversible interconversion of **1**/**2**/**3** could be repeated successfully by the addition of constant amounts of cys and Hg^{2+} to the solution of **2** suggesting **2** to be a suitable candidate for the *On-Off* probe for cys (Fig. 6). Moreover, the combined fluorescent behaviour of **1**/**2**/cys could be applied for the reversible and reproducible chemical switching represented via a molecular "INHIBIT" logic gate, employing Hg^{2+} and cys as two inputs and emission of **1** at 520 nm as the output, for which the *truth table* and symbol are shown in figure 7. In the *truth table*, the characters 0 and 1 indicate *off* and *on* states, respectively. The INHIBIT logic gates find applications in the subtractors, comparators and multiplexer logic circuits with the processing capabilities.[15]

Further, the coordination based selectivity of **1** for Hg^{2+} in solution, as discussed above, prompted us to mimic the sensing event in soft material (hydrogels). Thus, doping of the sodium cholate with various metal ions led to the corresponding cholate hydrogels (Fig. 8).[16] When the doped hydrogels were exposed to the solution of **1** at room temperature, only the gel network

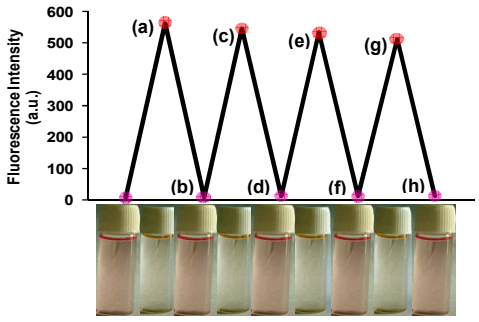


Fig. 6 Fluorescence intensity of **2** (1.64 x 10^{-5} M) upon alternate addition of cys and Hg^{2+} respectively: cys:Hg^{2+} in **(a)** 0:11.4 μM **(b)** 2.85:11.4 μM **(c)** 2.85:22.8 μM **(d)** 5.7:22.8 μM **(e)** 5.7:34.2 μM **(f)** 8.55:34.2 μM **(g)** 8.55:45.6 μM **(h)** 11.4:45.6 μM, and the corresponding color changes.

(a)

Input A (Hg^{2+})	Input B (cys)	Output λ_{em} 520 nm
0	0	0
1	0	1
0	1	0
1	1	0

(b)

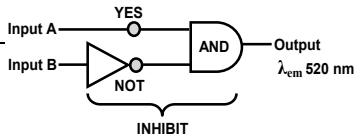


Fig. 7 (a) Truth table (b) symbol for the INHIBIT logic gate.

of the Hg^{2+} doped hydrogel collapsed entirely to result in fluorescent coloured sol phase (Fig. 8). On the basis of this observation, we propose that **1** can be used for detecting Hg^{2+} in the soft materials and can also offer itself for possible decontamination of Hg^{2+} contaminated soft materials based on metal cholate hydrogels (a chemical decontamination method). While adding solution of cys to the hydrogel containing **1** and Hg^{2+}, the fluorescence of the Hg^{2+} doped sol disappeared to replicate the colour changes depicted in Fig. 6, leading to qualitative sensing of cys in the hydrogel.

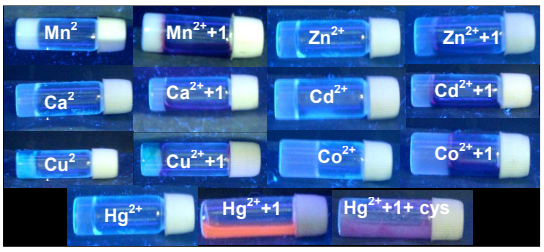


Fig. 8 Photographs of the metal cholate hydrogels before and after treatment with **1** under UV light and after addition of cys in the sol Hg^{2+}+**1**.

Conclusions

In summary, the *in situ* formed BODIPY dye:Hg^{2+} complex detects cys over other non-thiol amino acids. The reversible sensing event involving decomplexation/complexation has been supported by various spectroscopic data. Additionally, the possible use of the crown-BODIPY dyad to detect Hg^{2+} as well as cys from the soft materials operating via gel-sol conversion has been demonstrated.

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Notes and references

[a]*Department of Chemistry, Centre of Advanced Studies-I, Guru Nanak Dev University, Amritsar- 143 005, India.*
Tel: +91-183-2258853; Fax: +91-183-2258819-20.
Email: paramjit19in@yahoo.co.in (P. Kaur);
kamaljit.chem@gndu.ac.in (K. Singh).

† Electronic Supplementary Information (ESI) available: additional absorption, emission, Spectral and Gaussian data. See DOI: 10.1039/c000000x/

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A dioxadithiaazacrown ether-BODIPY dyad Hg^{2+} complex for detection of L-cysteine: Fluorescence switching and application on soft material

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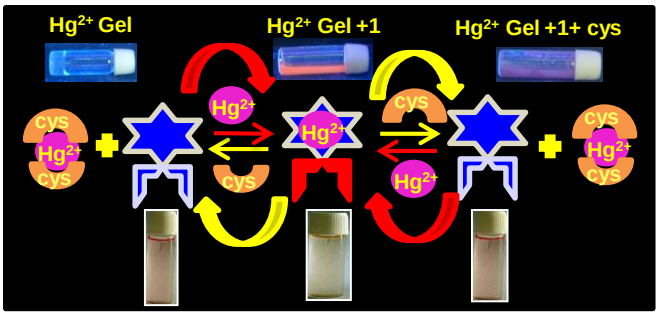

A Hg^{2+} coordinate complex of a 1,4-dioxa-7,13-dithia-10-azacyclopentadecane-BODIPY dyad recognises L-cysteine (cys) via reversible decomplexation/complexation and show switching of fluorescence upon sequential addition of cys and Hg^{2+} in solution as well as in hydrogel.