Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Supplementary Materials

Water Soluble Perylene Bisimide and its Turn Off/On Fluorescence are Used to Detect of Cysteine and Homocysteine

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1. Photos of PM12 solution after addition of different metal ions:

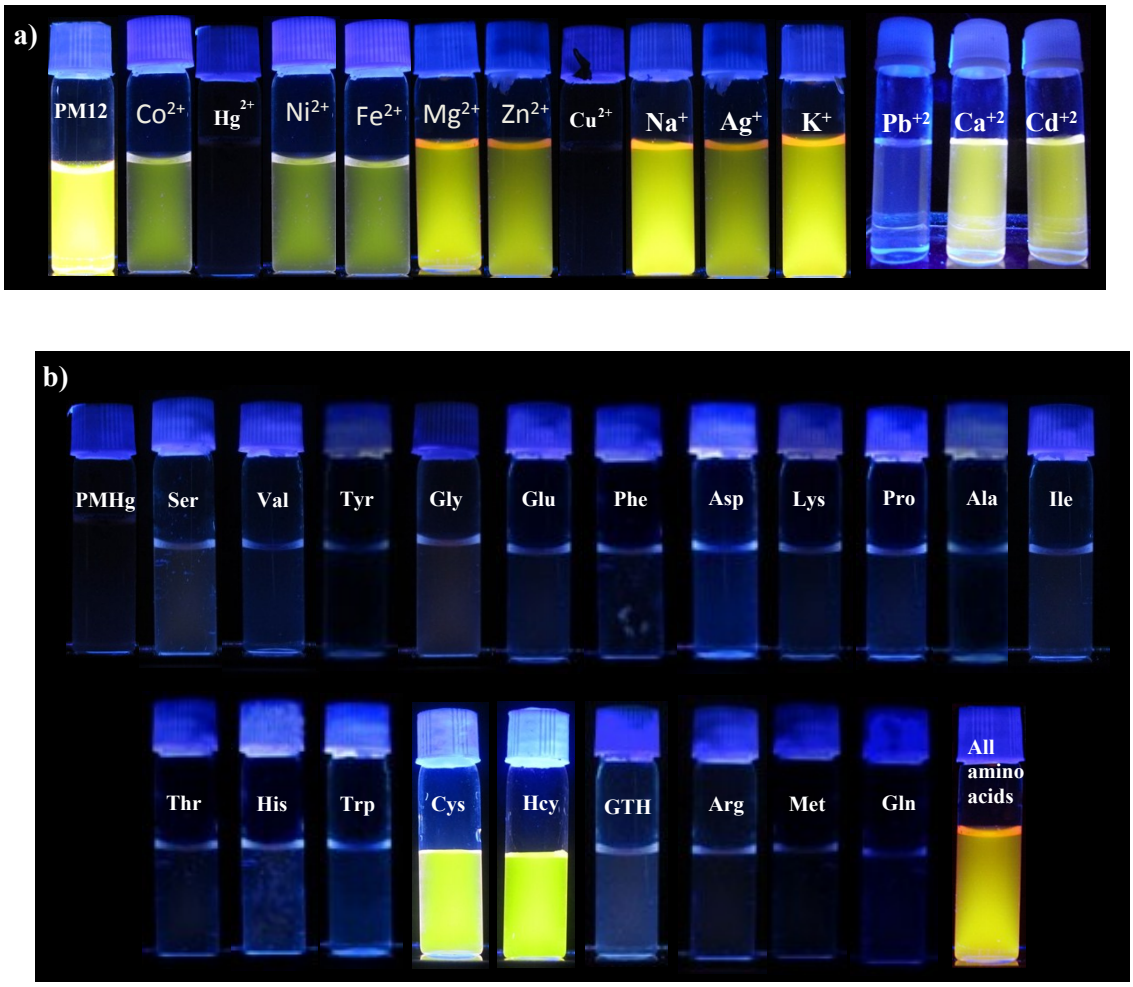


Fig. S1 (a) Photos of **PM12** (10 μM) solution and after addition of different metal ions at a concentration 1 mM under UV light at 365 nm excitation. (b) Photos of **PMHg**, **PMHg**-with all other amino acids under UV light at 365 nm [concentration of all amino acids 2 mM).

2. UV-Vis spectra of PM12 solution with addition of different metal ions:

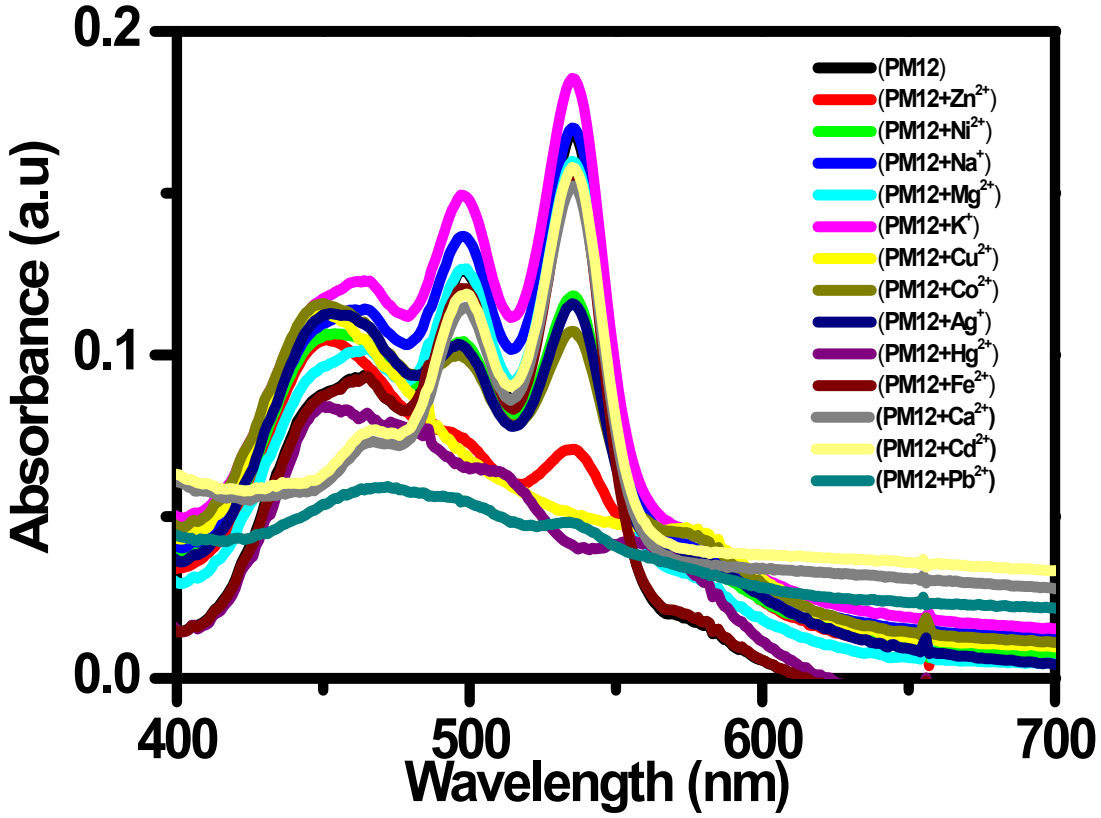


Fig. S2 UV-Vis spectra of **PM12** solution upon addition of 2 equivalent of Hg^{2+} and other various metal ions. [**P**] = 1×10^{-5} M, [**M**] = 2×10^{-5} M [pathlength = 10mm].

3. UV-Vis titration spectra of PM12 solution with addition of Hg^{2+} ion:

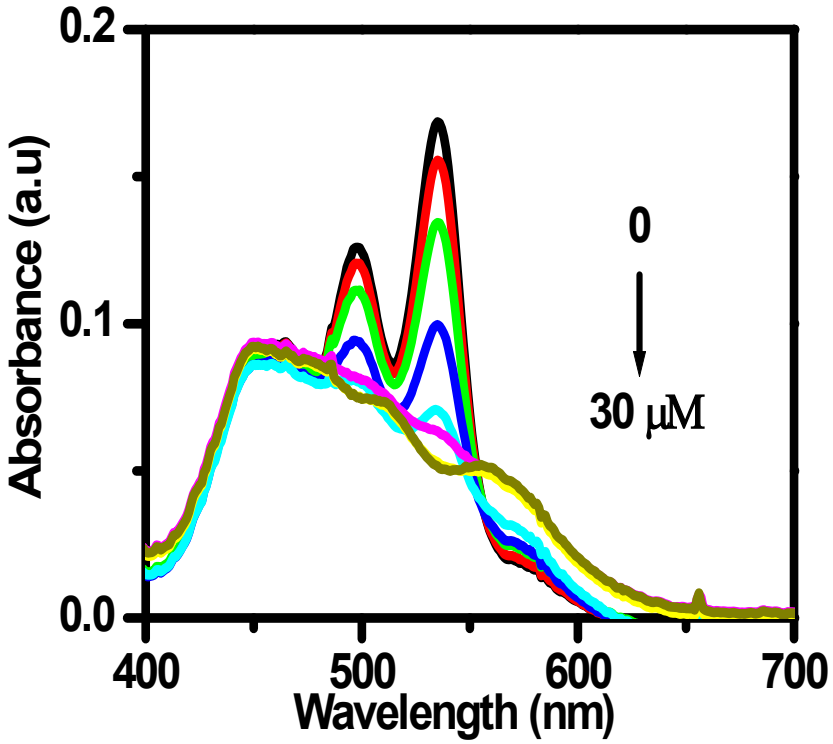


Fig. S3 UV-Vis titration spectra of **PM12** with Hg^{2+} in 0.1mM HEPES buffer solution. [**P**] = 1 × 10^{-5} M, [**M**] = 2 × 10^{-5} M [pathlength = 10mm].

4. Fluorescence spectra of PM12 solution with addition of different metal ions:

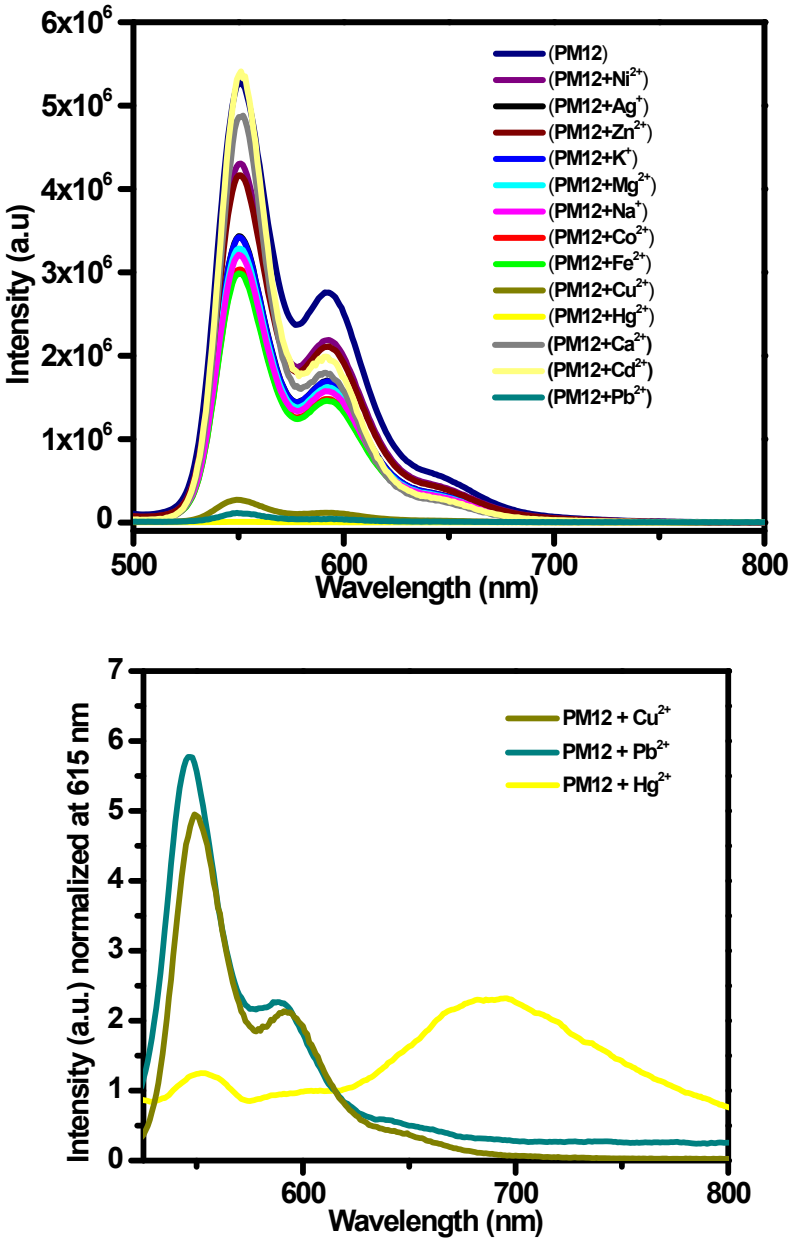


Fig. S4 a) Fluorescence spectra of **PM12** with all dications in 0.1mM HEPES buffer solution. [**P**] = 1 × 10^{-5} M, [**M**] = 2 × 10^{-5} M [pathlength = 10mm]. The concentrations of all other metal ions are 1 mM. b)A plot of normalllized instensity vs wavelength for three metal ions is showing the presence of peak at 690 nm for Hg^{+2} ion only. It indicates that quenching of fluorescence intensity is due to the formation of excimer. However, quenching of fluorescence intensity for Cu(II) and Pb(II) is due to heavy metal effect.

5. Fluorescence intensity ratio vs concentration of Hg^{2+} at 551 nm:

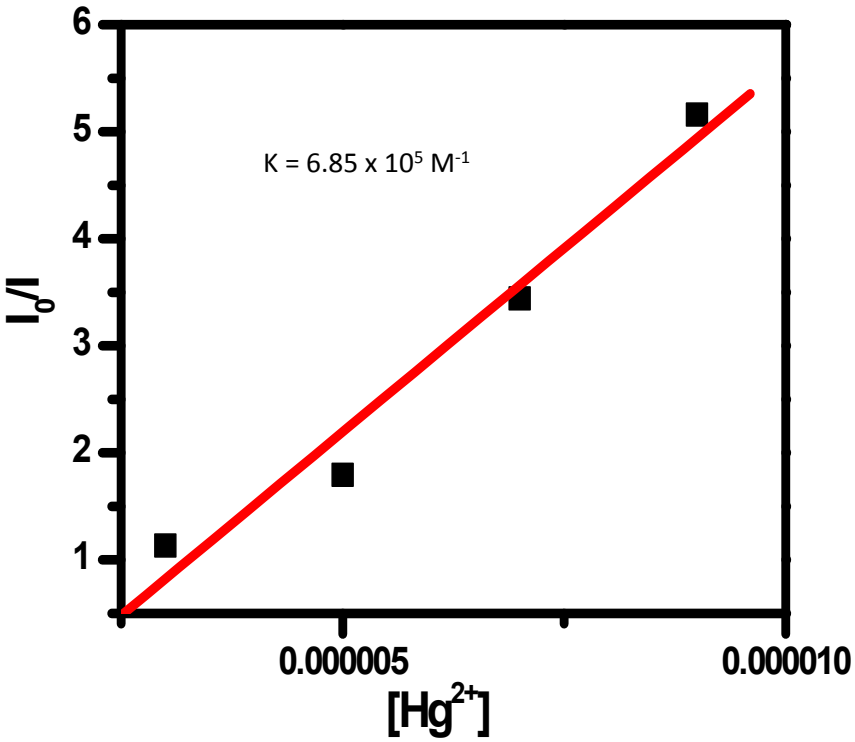


Fig. S5 Plot of fluorescence intensity ratio vs concentration of Hg^{2+} at 551 nm.

6. Fluorescence spectra of PMHg with addition of all amino acids and peptides:

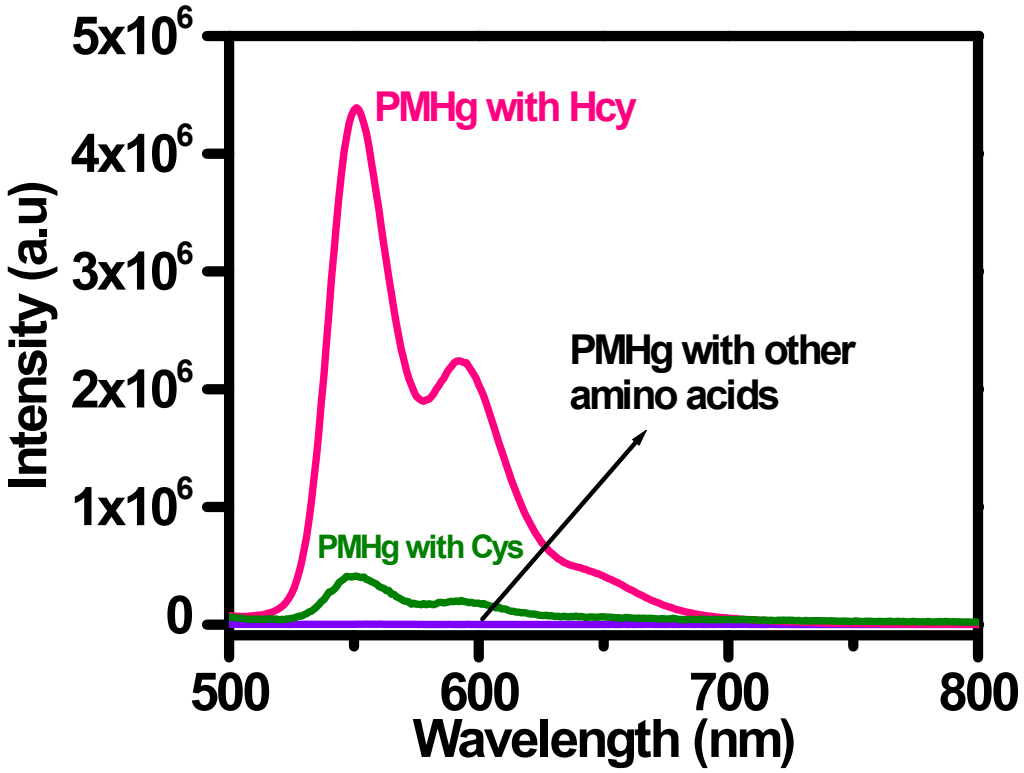
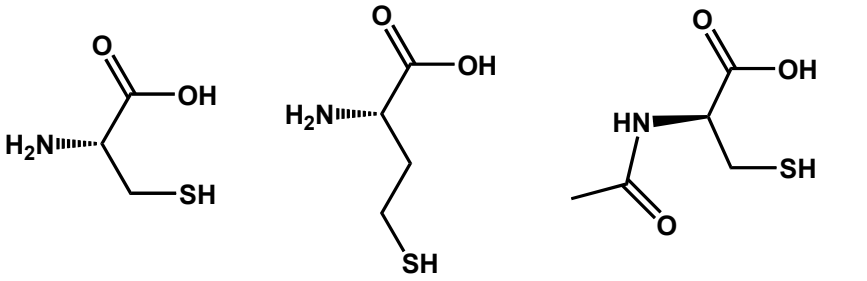
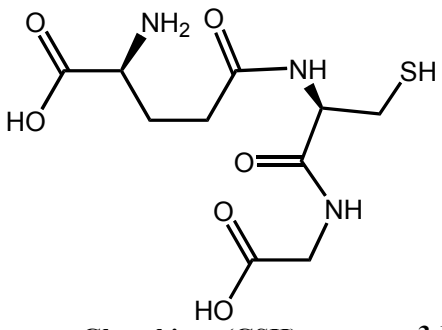


Fig. S6 a) Fluorescence spectra of PMHg (10 μM) upon addition all amino acids (2 mM) [λ_{ex} = 470 nm, pathlength = 10 mm].

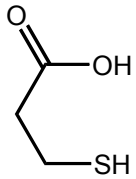
7. Fluorescence responses of thiol-containing compounds:



Cysteine (Cys) **Homocysteine (Hcy)** **N-Acetyl-L-cysteine (NAC)**



Glutathione (GSH)



3-Mercaptopropanoic acid (MPA)

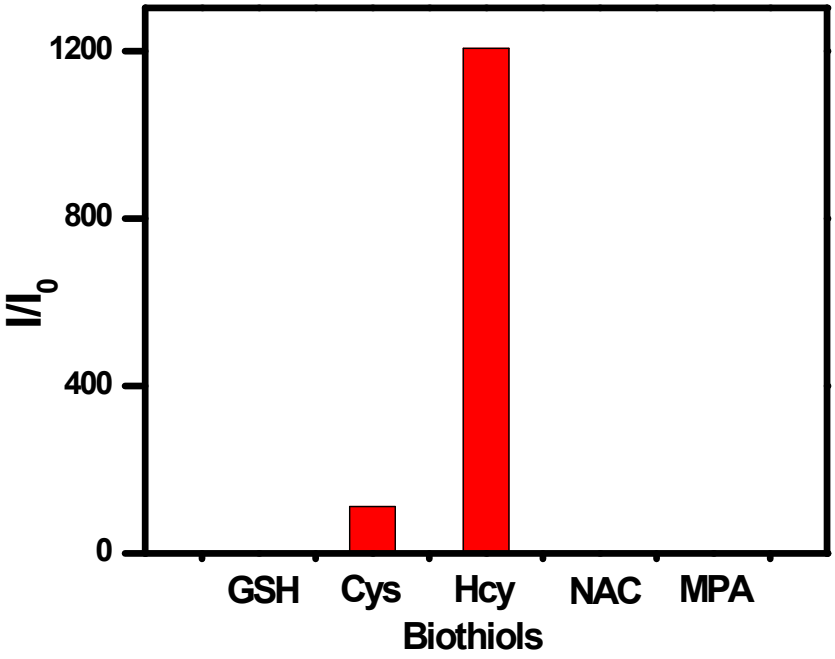


Fig. S7: (a) Chemical structure of thiol-containing compounds and (b) fluorescence responses of PMHg. The bars represent the integrated emission of PMHg (10 μM) in the presence 100 equivalent of different thiols. Other thiol containing compounds (N-acetyl-L-cysteine(insoluble in water), glutathione (bulky in nature), 3-mercapto-propanoic acid) do not have any interference.

8. Plot of change of emission intensity with cysteine added:

a)

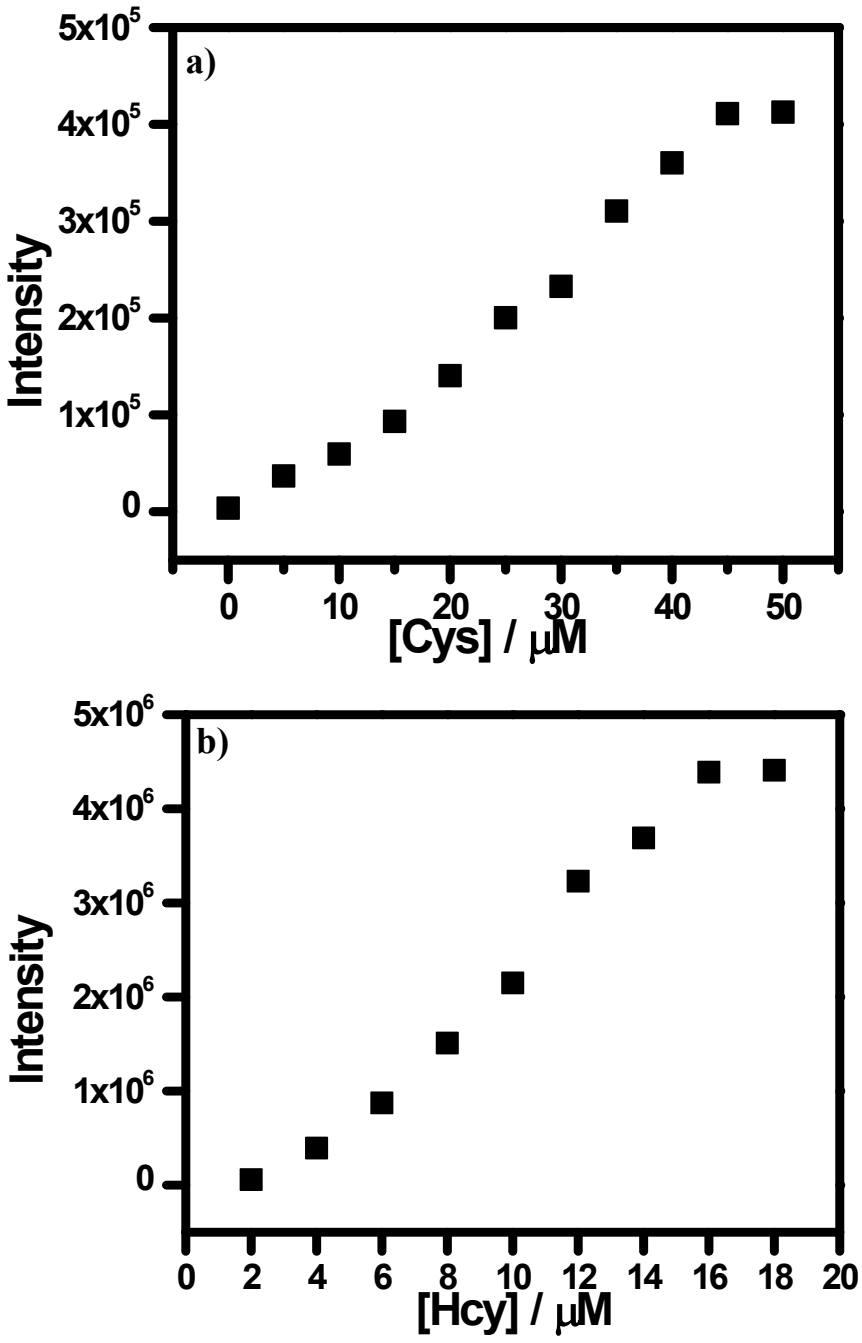


Fig. S8 Plot of change of emission intensity at 551 nm with the concentration of cysteine (a) and homocysteine (b) added to PMHg (1 x 10^{-5} M) in HEPES buffered (pH 7.0).

9. Benesi-Hildebrand analysis of cysteine/homocysteine added:

Benesi-Hildebrand analysis for determination stochiometry and binding constant between PMHg and Cysteine/Homocysteine:

Binding constants were estimated from titration experiments where data were plotted and the ratio between the *y*-intercept and the slope of the linear line of the "best fit" were obtained. The 1:1 interaction between **PMHg** and cysteine/homocysteine were analyzed according to the Benesi-Hildebrand equation for spectro fluorometric titrations:

$$\frac{1}{I_0 - I} = \frac{1}{I_0 - I'} + \frac{1}{K_a(I_0 - I')[object]}$$

where, K_a, I_0, I and I' are the (*i*) binding constant, the (*ii*) observed fluorescence intensity in the absence of analyte, the (*iii*) observed fluorescence intensity in the presence of analyte and the (*iv*) fluorescence intensity of the probe-analyte complex, respectively.

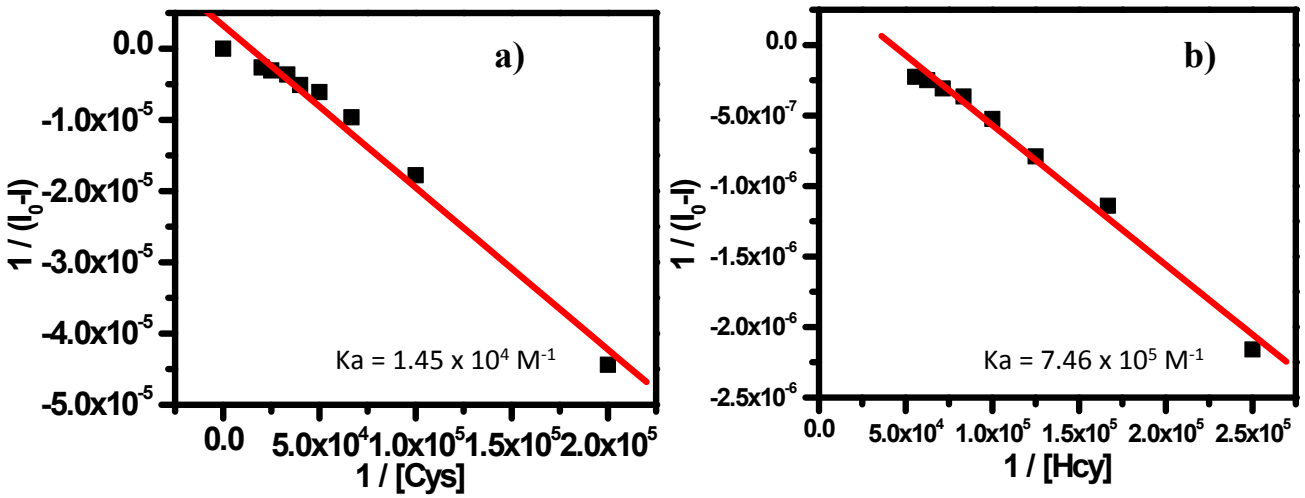


Fig. S9 Benesi-Hildebrand plots for the properly assigned 1:1 binding mode of fluorescence titration of PMHg (10 μM) with a) cysteine and b) homocysteine. Spectra were acquired after 30 min of each separate addition of cysteine and homocysteine.

10. Reversibility of the fluorescence with cysteine / homocysteine:

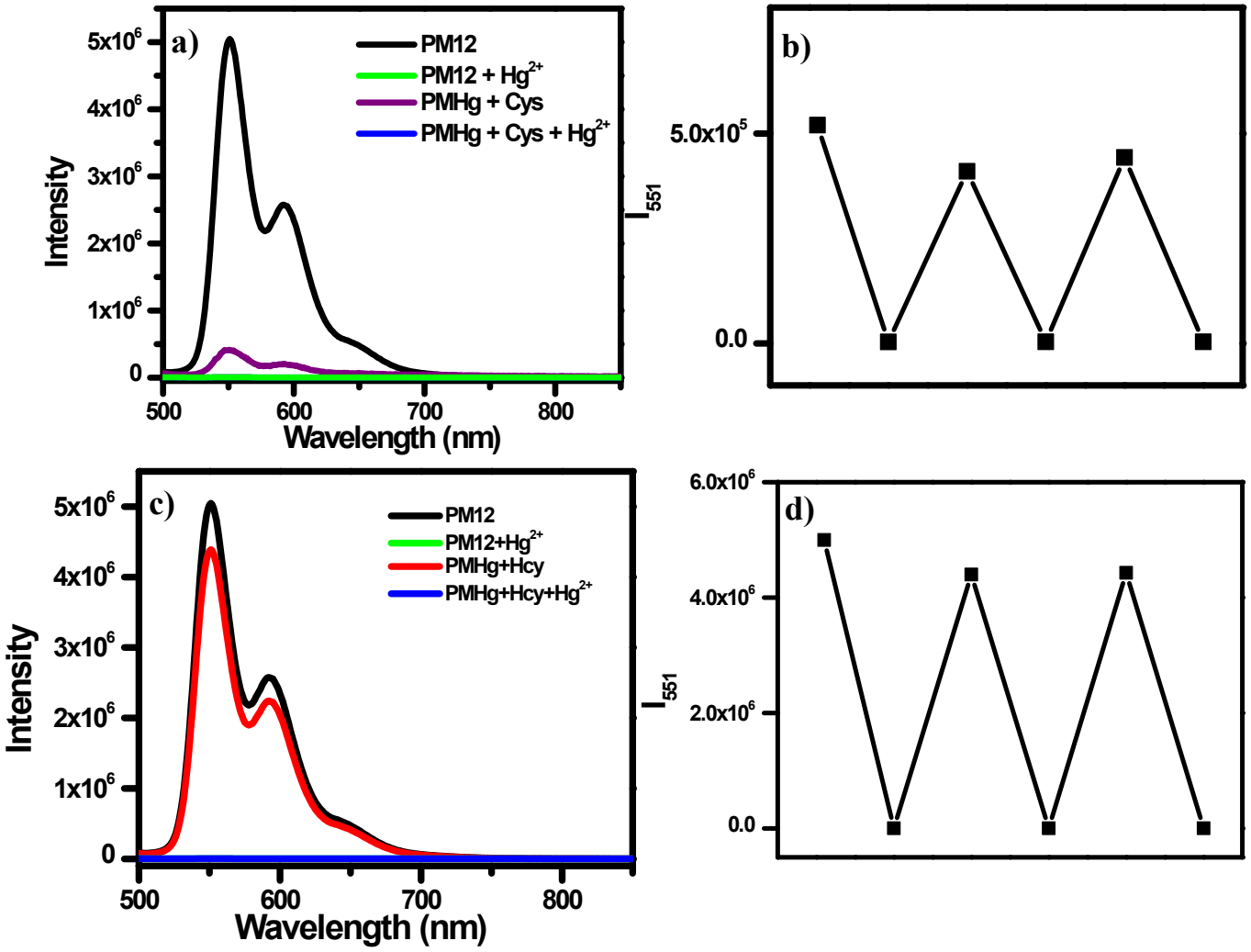


Fig. S10 Reversibility of fluorescence for **PMHg** in HEPES buffered upon alternate addition of mercury ions and (a-b) cysteine and (c-d) homocysteine. Initially, **PM12** having 10 μM concentration was taken (mother solution). Then Hg^{2+} was added of 2 equivalents to the mother solution. Cys and Hcy were added also 2 equivalents to the mother solution. This was followed repeatedly.

11. Fluorescence spectra of PMHg in diluted Serum or Urine sample with spiked Cysteine:

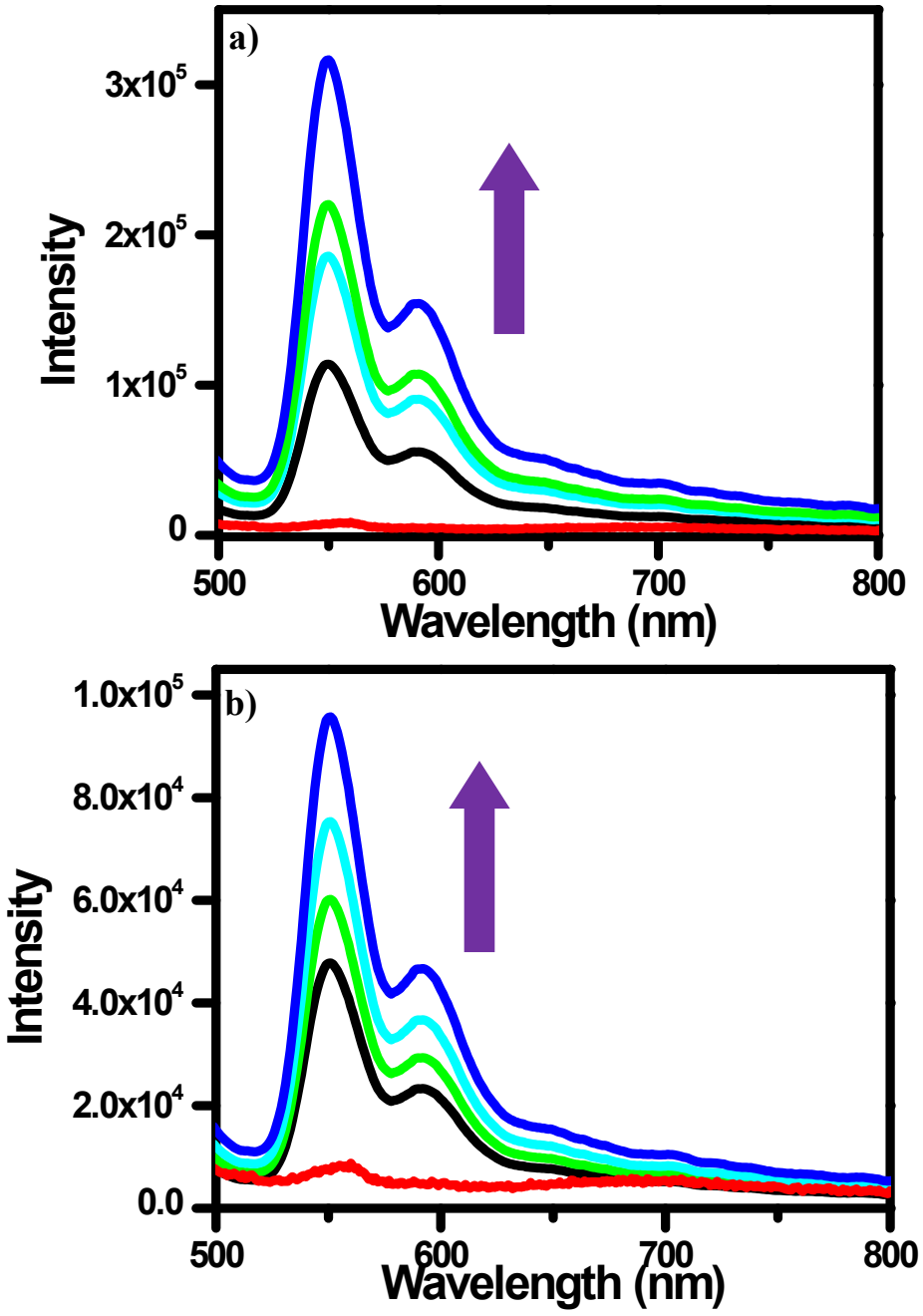


Fig. S11 Fluorescence spectra of **PMHg** (10 μM) (a) diluted serum samples (20-fold diluted) with spiked Cys. Arrow indicates the gradual addition of spiked Cys/Hcy from bottom to top, the spiked Cys levels are: 0 μM, 5 μM, 12 μM, 15 μM, 23.25 μM. (b) Fluorescence spectra of diluted urine samples (10-fold diluted) with spiked Cys. As pointed by the arrow, from top to bottom, the spiked Cys levels are: 0 μM, 2.5 μM, 5 μM, 7.5 μM, 10 μM.

12. Comparison of fluorescence intensity between that of PMHg in diluted serum and urine sample:

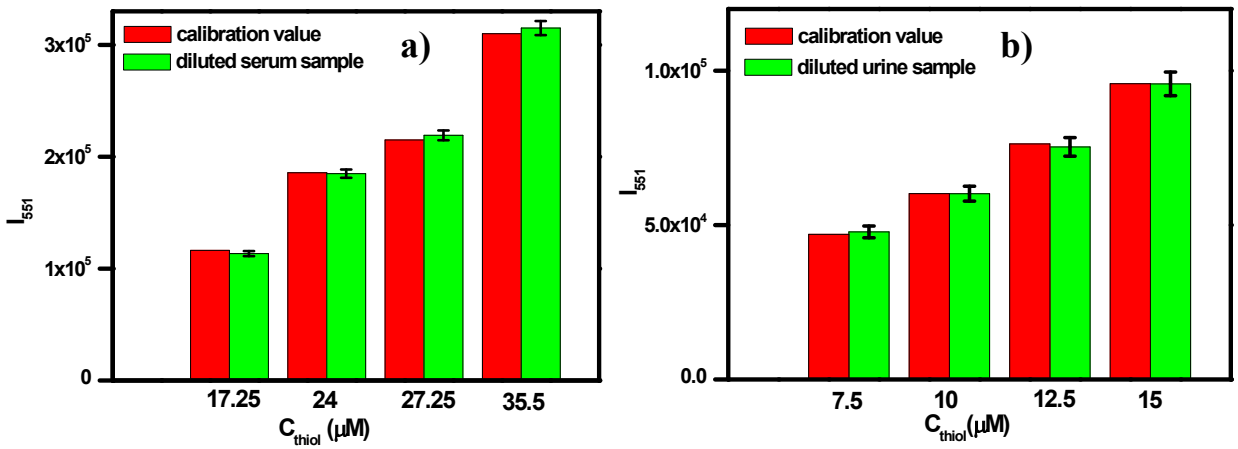


Fig. S12 a). Comparison of fluorescence intensity between that of **PMHg** (10 μM) in diluted serum with error bar (diluted by pH 7.0 HEPES buffered water) and that obtained from the Cys calibration curve at different thiol concentrations (the orignailly-existing thiol plus spiked Cys). (λ_{exc} = 470 nm). **Red column:** the fluorescence intensity values obtained from the calibration curve (as shown in **Fig. S11a**) at different concentrations. The C_{thiol} concentrations represent the thiol (Cys) added into the HEPES buffered (pH 7.0) water. **Green column:** the fluorescence intensity for the diluted serum samples of varied thiol concentrations (the determined original thiol concentration plus varied spiked thiol concentrations). In the diluted serum sample without spiking Cys, it is determined that there are 12.25 μM thiols in the test solution (which means the concentration of thiols in the undiluted serum is ca. 245 μM). The C_{thiol} concentrations (17.25, 24, 27.25, 35.5 μM) represent the total thiols concentration in the test samples, namely the 12.25 μM thiols plus the spiked Cys amounts (5, 11.75, 15, 23.25 μM).

b) Comparison of fluorescence intensity between that of **PMHg** (10 μM) in diluted urine with error bar (diluted by pH 7.0 HEPES buffered water) and that obtained from Cys calibration curve at different thiol concentrations (the orignailly-existing thiol plus spiked Cys). (λ_{exc} = 470 nm). **Red column:** the fluorescence intensity ratio values obtained from the Cys calibration curve (as shown in **Fig. S11b**) at different concentrations. The C_{thiol} concentrations represent the thiol (Cys) added into the HEPES buffered (pH 7.0) water. **Green column:** the fluorescence intensity for the diluted urine samples of different thiols concentrations (the determined original thiol concentration plus the varied spiked thiol concentrations). In the diluted urine sample without spiking Cys, it is determined that there are 5 μM thiols in the test solution (which means the concentration of thiols in the undiluted urine is ca. 50 μM). The C_{thiol} concentrations (7.5, 10, 12.5, 15 μM) represent the total thiols concentration in the test samples, namely the 5 μM thiols plus the spiked Cys amounts (2.50, 5, 7.50, 10 μM).

13. Table for the determination of cysteine in dilute serum and urine sample:

Table S1. Determination of endogenous thiols and that plus spiked Cys levels in serum (20-fold diluted) using PMHg as the probe.

Sample	Determined biothiol[(a)] (10^{-6} M)	Added Cys (10^{-6} M)	Combined thiol (10^{-6} M)	Measured (10^{-6} M)	Recovery (%)
1	12.25	-	-	-	-
2		5	17.25	17.17	99.53
3		11.75	24	23.77	99
4		15	27.25	27.52	100.9
5		23.25	35.5	35.35	99.5

Note: (a) the determined biothiol value in the diluted serum sample without spiking Cys is 12.25 **μM**, **which means the biothiols concentration in undiluted serum is ca. 245 μM**. Cys calibration curve was used as the standard.

Table S2. Determination of endogenous thiols and that plus spiked Cys levels in human urine (10-fold diluted) using PMHg as the sensor.

Sample	Determined biothiol[(a)] (10^{-6} M)	Added Cys (10^{-6} M)	Combined thiol (10^{-6} M)	Measured (10^{-6} M)	Recovery (%)
1	5	-	-	-	-
2		2.5	7.5	7.47	99.6
3		5	10	9.62	96.2
4		7.5	12.5	12.40	99.2
5		10	15	14.51	96.73

Note: (a) The determined biothiol value in the diluted urine sample without spiking Cys is 5 μM, **which means the biothiol concentration in undiluted urine sample is ca. 50 μM**. Cys calibration curve was used as the standard.

14. Fluorescence spectra of PMHg in diluted Serum or Urine sample with spiked homocysteine:

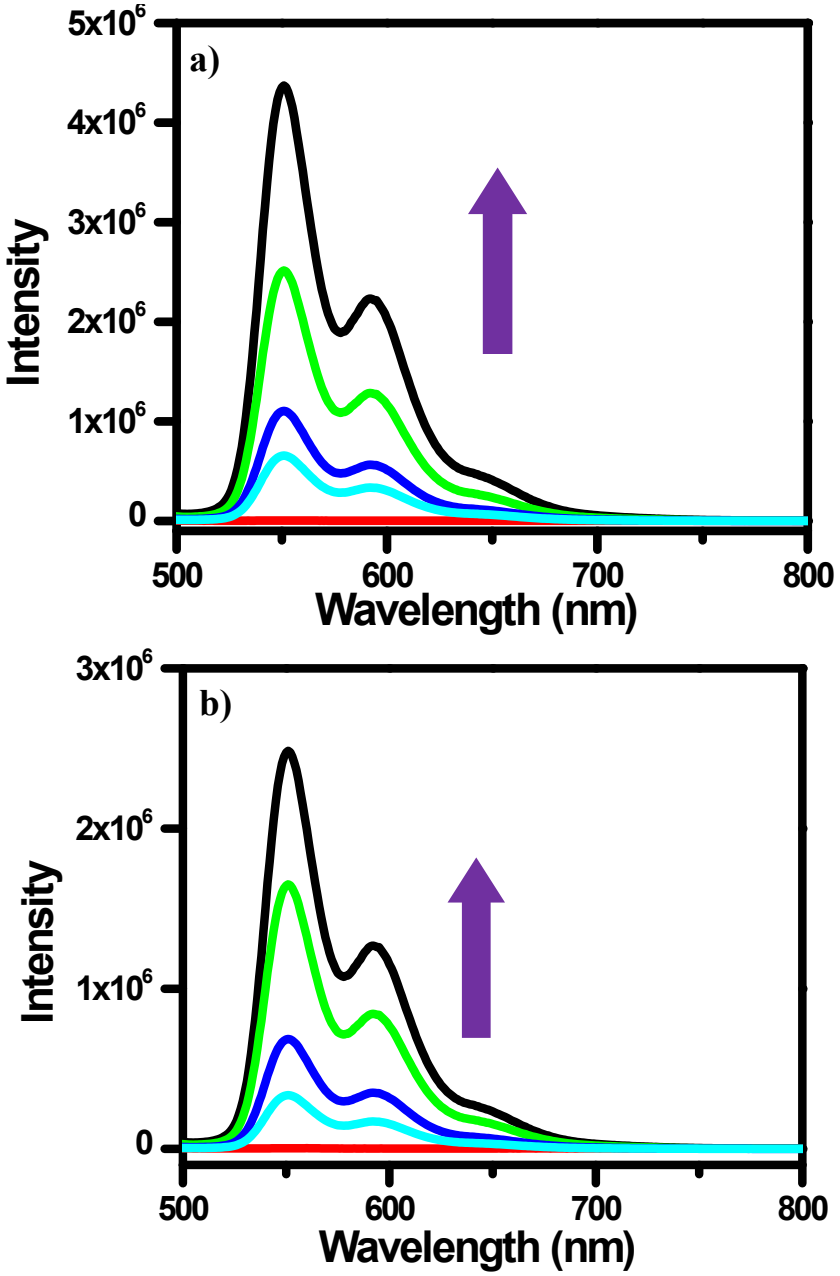


Fig. S13 Fluorescence spectra of **PMHg** (10 μM) in diluted serum samples (10-fold diluted) with spiked Hcy. As pointed by the arrow, from bottom to top, the spiked Hcy levels are: 0 μM, 2 μM, 5.5 μM, 9 μM, 12.5 μM. (b) Fluorescence spectra of diluted urine samples (10-fold diluted) with spiked Hcy. As pointed by the arrow, from top to bottom, the spiked Hcy levels are: 0 μM, 2.50 μM, 4 μM, 7.25 μM, 10 μM.

15. Comparison of fluorescence intensity between that of PMHg in diluted serum and urine sample:

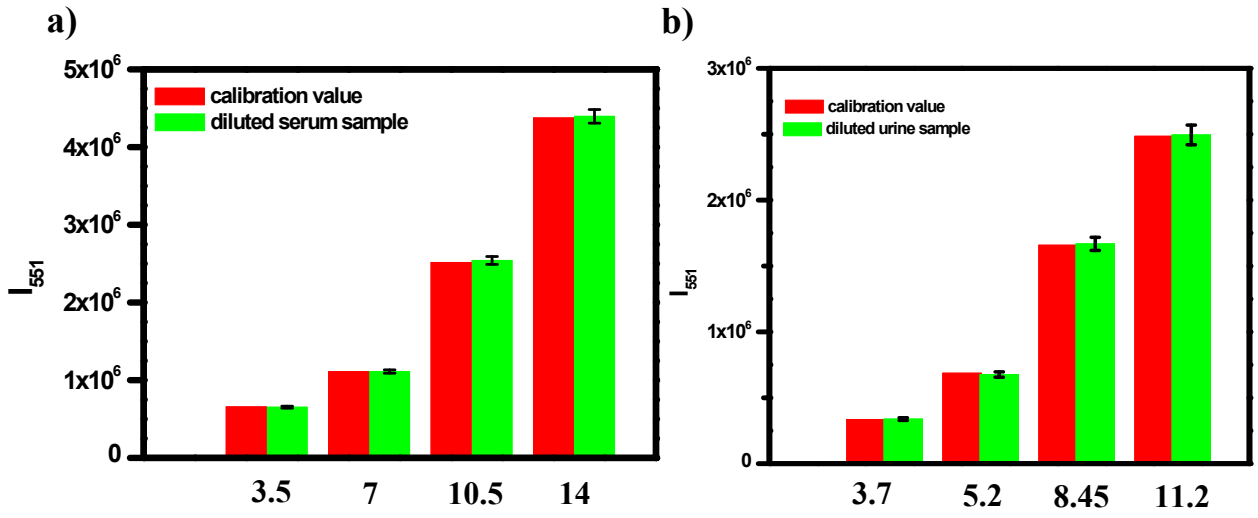


Fig. S14 (a) Comparison of fluorescence intensity between that of PMHg (10 μM) in diluted serum with error bar (diluted by pH 7.0 HEPES buffered water) and that obtained from the Hcy calibration curve at different thiol concentrations (the orignailly-existing thiol plus spiked Hcy). (λ_{exc} = 470 nm). **Red column:** the fluorescence intensity values obtained from the calibration curve (as shown in **Fig. S13a**) at different concentrations. The C_{thiol} concentrations represent the thiol (Hcy) added into the HEPES buffered (pH 7.0) water. **Green column:** the fluorescence intensity for the diluted serum samples of varied thiol concentrations (the determined original thiol concentration plus varied spiked thiol concentrations). In the diluted serum sample without spiking Hcy, it is determined that there are 1.5 μM thiols in the test solution (which means the concentration of thiols in the undiluted serum is ca. 15 μM). The C_{thiol} concentrations (3.5, 7, 10.5, 14 μM) represent the total thiols concentration in the test samples, namely the 1.5 μM thiols plus the spiked Hcy amounts (2, 5.5, 9, 12.5 μM).

(b) Comparison of fluorescence intensity between that of PMHg (10 μM) in diluted urine with error bar (diluted by pH 7.0 HEPES buffered water) and that obtained from Hcy calibration curve at different thiol concentrations (the orignailly-existing thiol plus spiked Hcy). (λ_{exc} = 470 nm). **Red column:** the fluorescence intensity values obtained from the Hcy calibration curve (as shown in **Fig. S13b**) at different concentrations. The C_{thiol} concentrations represent the thiol (Hcy) added into the HEPES buffered (pH 7.0) water. **Green column:** the fluorescence intensity for the diluted urine samples of different thiols concentrations (the determined original thiol concentration plus the varied spiked thiol concentrations). In the diluted urine sample without spiking Hcy, it is determined that there are 1.2 μM thiols in the test solution (which means the concentration of thiols in the undiluted urine is ca. 12 μM). The C_{thiol} concentrations (3.7, 5.2, 8.45, 11.2 μM) represent the total thiols concentration in the test samples, namely the 1.2 μM thiols plus the spiked Hcy amounts (2.50, 4, 7.25, 10 μM).

16. Table for the determination of homocysteine in dilute serum and urine sample:

Table S3. Determination of endogenous thiols and that plus spiked Hcy levels in serum (10-fold diluted) using PMHg as the probe.

Sample	Determined biothiol[(a)] (10^{-6} M)	Added Hcy (10^{-6} M)	Combined thiol (10^{-6} M)	Measured (10^{-6} M)	Recovery (%)
1	1.5	-	-	-	-
2		2	3.5	3.52	100.5
3		5.5	7	7.07	101
4		9	10.5	10.56	100.5
5		12.5	14	13.95	99.6

Note: (a) the determined biothiol value in the diluted serum sample without spiking Hcy is 1.5 **μM**, **which means the biothiols concentration in undiluted serum is ca. 15 μM**. Hcy calibration curve was used as the standard.

Table S4. Determination of endogenous thiols and that plus spiked Hcy levels in human urine (10-fold diluted) using PMHg as the sensor.

Sample	Determined biothiol[(a)] (10^{-6} M)	Added Hcy (10^{-6} M)	Combined thiol (10^{-6} M)	Measured (10^{-6} M)	Recovery (%)
1	1.2	-	-	-	-
2		2.5	7.5	7.51	100.1
3		4	5.2	5.22	100.3
4		7.25	8.45	8.48	100.3
5		10	11.2	11.01	98.4

Note: (a) The determined biothiol value in the diluted urine sample without spiking Hcy is 1.2 μM, **which means the biothiol concentration in undiluted urine sample is ca. 12 μM**. Hcy calibration curve was used as the standard.

17a. MTT Assays

MTT assay was performed to evaluate the effect **P** and **PM12** on viability of HeLa cells.[2] For this purpose HeLa cells were cultured at 37°C in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FBS, 100 U/ml penicillin and 100μg/ml streptomycin in a 5% CO_2 humidified environment. Cells were then seeded at a density of 10,000 cells per well on 96-well plates in 100μl medium and incubated for 24 h. After incubation, the culture media was replaced with fresh media containing **P** (20 μM) and **PM12** and HeLa cells were further incubated for 24 hrs. After this, 10μl of a 5 mg/ml MTT solution was added to each well and the incubation was continued for 4hrs. Finally, 100μl of a solution containing 50% dimethylformamide and 20% SDS (pH 4.7) was added to each well and incubated for overnight. After overnight incubation the absorption values at 560nm were measured using an automatic microtiter plate reader (Thermo Fisher Scientific).

19b **Fluorescence imaging of PM12/PMHg/ PMHg-Cys in HeLa cells**

To study the **PM12/PMHg/ PMHg-Cys** internalization in HeLa cells, cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FBS, 100U/ml penicillin and 100μg/ml streptomycin and seeded on a clean cover glass in 24 well tissue culture plate at a density of 10,000 cells/well in 1 ml culture media. After 24 h of incubation in a 5% CO_2 humidified environment at 37°C old media was discarded and aqueous solution of **PM12** (diluted in cell culture media to cells at a final concentration of 10 μM) was added to cells. After 0.5 h of incubation of HeLa cells with **PM12**, cells were gently washed with PBS to remove the excess and free **PM12**. After this cells were further incubated for 0.5 h with $HgCl_2$ (20 μM) in cell culture media, washed and imaged. HeLa cells incubated with only PM12 were also imaged simultaneously. In a parallel experiment, after incubation with $HgCl_2$ (0.5 h), cells were gently washed with PBS and incubated with cysteine solution (20 μM) in cell culture media. HeLa cells were further incubated for 0.5h before imaging them under confocal microscope (Carl Zeiss Micro Imaging GmbH, Germany).

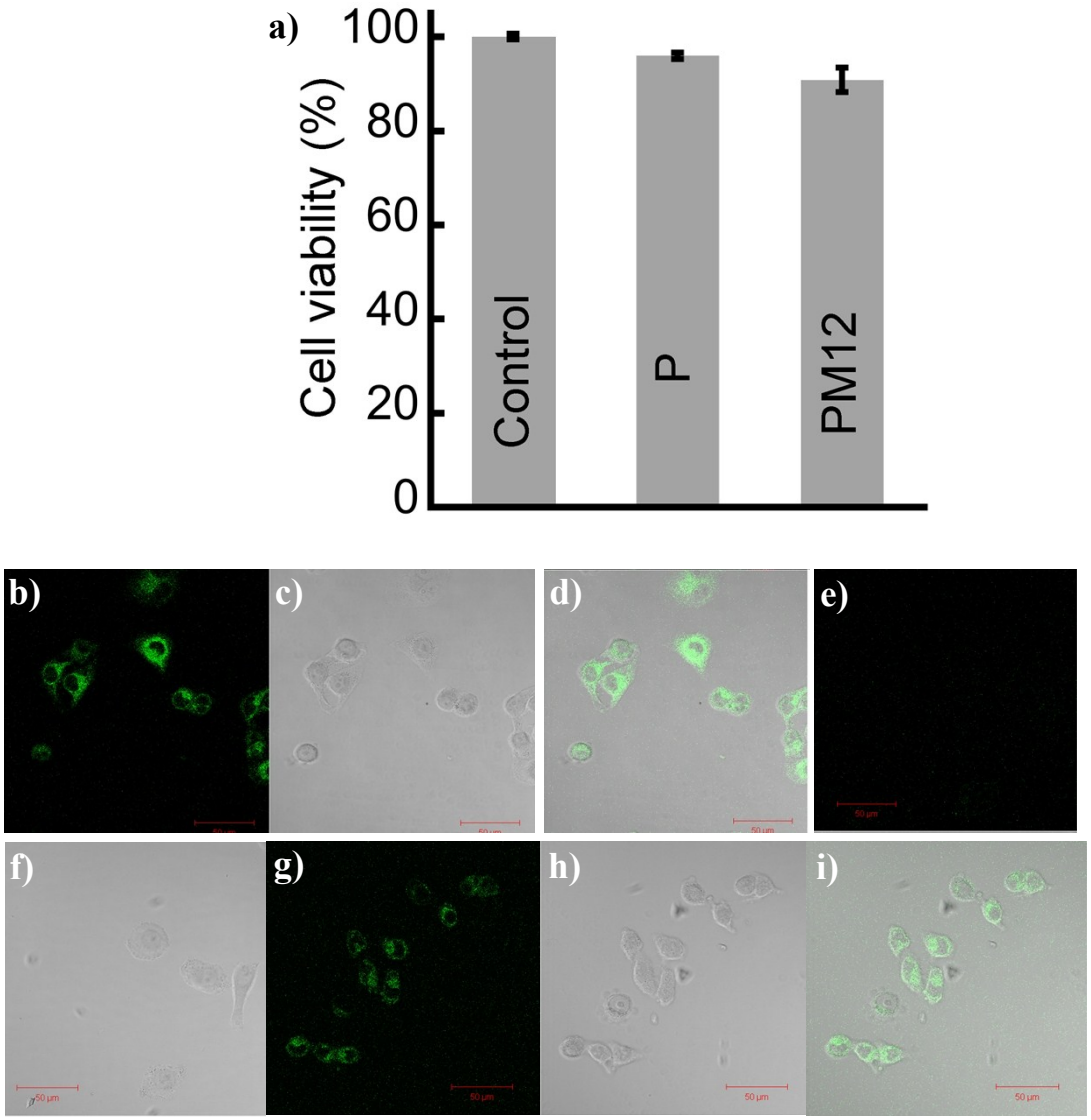


Fig. S15 (a) MTT assay of **P** and **PM12**. Confocal images of HeLa cells: (b-d) cells incubated with **PM12** (10 μM) for 0.5 h. (e-f) cells incubated with **PM12** (10 μM) for 0.5 h, then with $HgCl_2$ (20 μM) for 30 min. (g-i) cells incubated with **PM12** (10 μM) for 0.5 h, then with $HgCl_2$ (20 μM) for 30 min and again with Cys (20 μM). Bright field (c, f and h), fluorescence (b, e and g) and overlap field (d and i). Scale bars are 50μm.

18. Table for comparison of different fluorescence-based methods for Hg^{2+} detection:

Sl. No.	Publications	Dye derivatives	Medium	Bioimaging
1	*Inorg. Chem.*, 2008, *47*, 5169-5176.	Naphthalene	DMSO/H_2O	No
2	*Dalton Trans.*, 2012, *41*, 1801-1807.	BODIPY	CH_3CN/H_2O	No
3	*Org. Lett.*, 2011, *13*, 5028-5031.	Pyrene	H_2O/DMF	No
4	*Chem. Eur. J.*, 2012, *18*, 11188-11191.	Naphthalene	H_2O	No
5	*Org. Lett.*, 2011, *13*, 1162–1165.	Squaraine	AcOH/H_2O	No
6	*Chem. Commun.*, 2012, *48*, 8371-8373.	Naphthalene	DMSO/PBS buffer	Yes
7	*Nature Protocols*, 2007, *2*, 1740-1745.	Rhodamine	H_2O	Yes
8	*J. Mater. Chem.*, 2012, *22*, 478-482.	Perylene	THF	No
9	*ACS Appl. Mater. Interfaces*, 2012, *4*, 3657-3662.	Perylene	Acetone/BR buffer	Yes
10	*Chem. Commun.*, 2010, *46*, 4938-4940.	Perylene	DMF/H_2O	No
11	***Our Method***	Perylene	H_2O	Yes

19. Table for comparison of different methods for cysteine/homocysteine detection:

Sl. No.	Publications	Interference by other amino acids	Detection method	Reversible/Irreversible	Detection of Cys/Hcy in biological systems
1	*Angew. Chem. Int. Ed.*, 2011, *50*, 1-5.	No (need special technique of surfactant catalysis)	Addition/cyclization	Irreversible	No
2	*Chem. Commun.*, 2012, *48*, 1147-1149.	Yes	Nanoparticle	Not shown	No
3	*Chem. Commun.*, 2012, *48*, 2722-2724.	Yes	Michael addition	Irreversible	Yes
4	*Chem. Commun.*, 2012, *48*, 6007-6009.	Yes	Monomer-excimer transformation	Reversible	No
5	*J. Am. Chem. Soc.*, 2004, *126*, 438-439.	No	Cyclization	Irreversible	No
6	*J. Mater. Chem. B*, 2013, *1*, 438-442.	Yes	Michael addition	Irreversible	Yes
7	***Our method***	No	Chemosensing ensemble probe	Reversible	Yes

20. References:

1. V. Thomsen, D. Schatzlein and D. Mercuro, *Spectroscopy,* 2003, **18**, 112.

2. T. Mosmann, *J Immunol Methods,* 1983, **65**, 55.