A Novel and Cost Effective Isatin Based Schiff Base Fluorophore: Highly Efficient "Turn-Off" Fluorescent Sensor for the Selective Detection of Cysteine in Aqueous Medium

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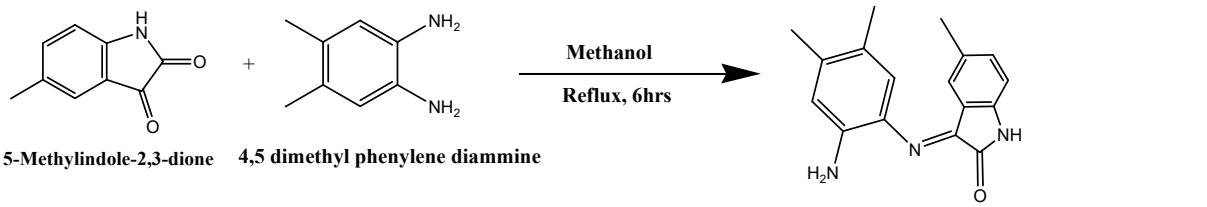
Scheme 1. Synthesis of MPD 5MI



5-Methylindole-2,3-dione **4,5 dimethyl phenylene diammine**

3-(2-Amino-4,5-dimethyl-phenylimino)-5-methyl-1,3-dihydro-indol-2-one

Orange yellow solid, Yield: 78%; m. p: 160-162 °C. UV–visible (λmax, DMSO, nm): 323 (π→π*), 438 (n→π*). Selected IR data (KBr) v/cm−1: 3118 (N-H str), 1595 (C=N str imine), 1624 (C=O str); 1 H-NMR (300 MHz, DMSO) δ (ppm): 8.05 (s, 1H), 7.81-7.86 (d, 1H), 7.47 (s,1H), 7.20 (s, 1H), 7.09 (s, 1H), 6.77 (s,1H), 6.16 (s, 1H), 2.52 (s, 3H), 2.41 (s, 3H), 2.25 (s, 3H),4.0 (s,1H); 13C-NMR (75 MHz, DMSO) δ(ppm): 164.30, 161.44, 138.44, 137.51, 136.85, 134.92, 133.06, 130.80, 129.53, 129.18, 123.75, 122.20, 120.27, 117.73, 20.35, 14.32, 14.05; ESI-MS(m/z): [M+1] = 280.01. Anal. Calc. for C17H17N3O: C, 73.10, H, 6.13, N, 15.04, O, 5.73. Found (%) C, 72.99, H, 6.01, N, 15.41, O, 5.01.

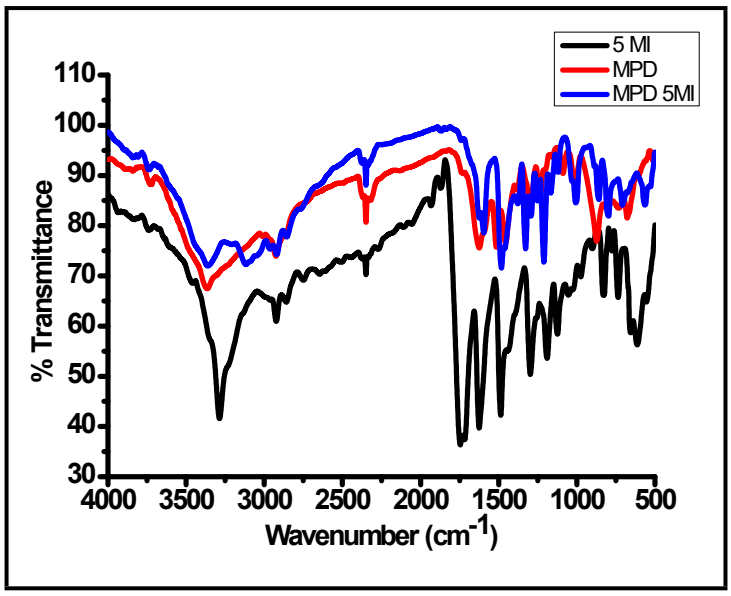


Fig S1. FT-IR Spectrum of 5-methylisatin (5MI, Black), 4,5 –dimethyl phenylene diamine(MPD, Red) and 2-Amino-4,5-dimethyl-phenylimino)-5-phenyl-1,3-dihydro-indol-2-one (MPD 5MI, blue)

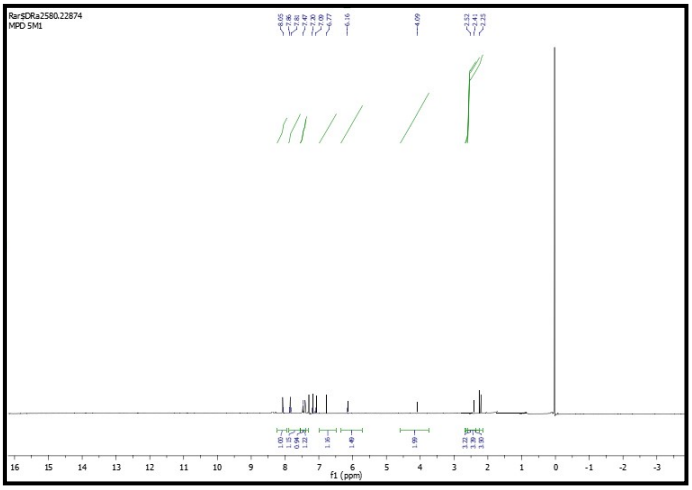


Fig S2. ^{1}H-NMR Spectrum of MPD 5MI

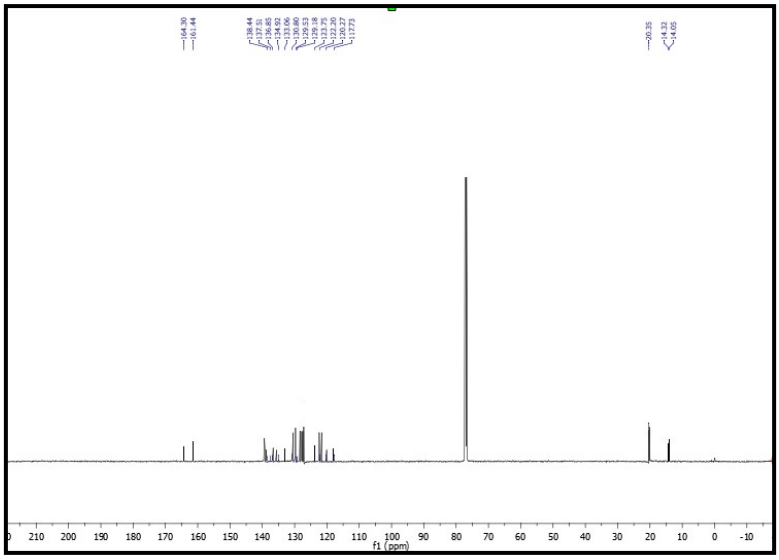


Fig S3. ^{13}C-NMR Spectrum of MPD 5MI

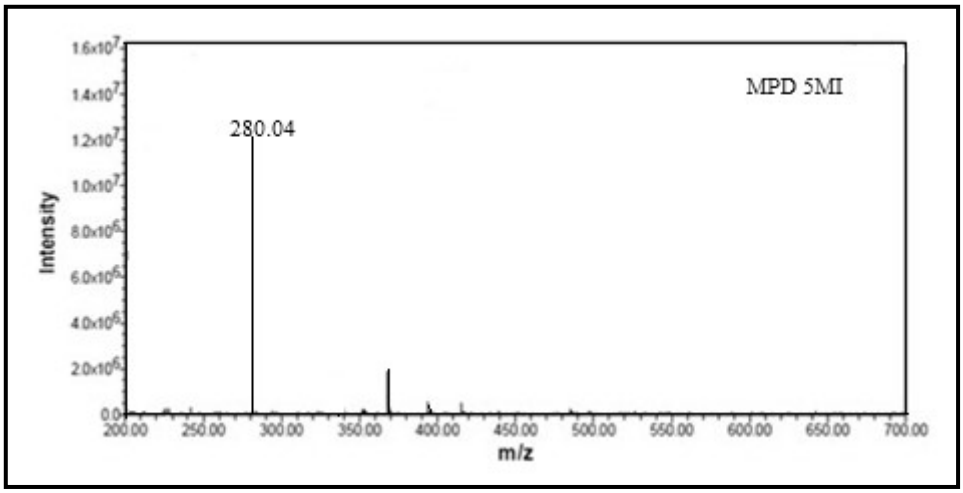


Fig S4. LC-MS Spectrum of MPD 5MI.

S2. Cyclic voltammetry measurements

To understand the basic electronic structure of the probe, HOMO, LUMO and bandgap were evaluated by Cyclic-Voltammetry measurements. In this regard, we have dissolved a desired amount of the sample in 10 ml of the acetonitrile solvent which we have chosen as the electrolyte. In this 0.1 M solution of tetra-n-butylamonium hexafluorophospahte (Bu_4NPF_6) was added. The electrodes including the glassy carbon electrode (working electrode), Ag/AgCl (auxiliary electrode) and the Pt wire (counter electrode) was dipped in to the mixture of solution and the measurements were done at a scan rate of 10 mV/s concerning ferrocene as the external standard.

S3. Computational method

The HOMO and LUMO orbitals of the molecules was elucidated by optimizing the geometry of molecules on Gaussian 03 package at density functional theory (DFT) level of theory based on B3LYP method along with the 6-31G (d,p) basis set [47]. The optimized structure of the probe was visualized using Gauss view version 5.0.9. Energy of the highest molecular orbital (E_{HOMO}) and the lowest molecular orbital (E_{LUMO}) and the corresponding band gap (ΔE,eV) between the E_{HOMO} and E_{LUMO} has been evaluated.

S4. Fluorescent sensing of Cys using MPD 5MI

Sensing capacity of MPD 5MI towards cysteine was evaluated, for that 100 μL of 1×10^{-3} M concentration of probe was mixed with various concentration of Cys ($5 \times 10^{-6} M$ to 4×10^{-7} M) and DMF was finally added to make total volume to 3 ml. All the analysis was carried out at a pH of 6. To carry out selectivity study, stock solutions of other interfering amino acids were prepared by dissolving it in aqueous solution in the above-mentioned concentration. Finally, fluorescence intensity was evaluated by exciting the samples at 400 nm, and also selectivity of Cys was determined by comparison with other amino acids under the same experimental conditions.

S5. Analysis of real samples.

Synthetic blood, urine was used to demonstrate the practical utility of MPD 5MI for the sensing of Cysteine. According to the literature, we have prepared synthetic blood and urine for real sample studies [48]. We have added various concentration of Cys in the range $5 \times 10^{-6} M$ to 4×10^{-7}M. Fluorescence were monitored. The recovery percentage and relative standard deviation was evaluated.

S6. Quantum Yield of MPD 5MI

Determination of QY: It is defined as the ratio of number of emitted photons to the number of absorbed photons. It can be calculated by using the equation:

$$QY\ compound = QY\ reference \times \frac{m}{mr} \times \frac{\eta}{\eta r} \qquad \text{Eqn (1)}$$

Where, mr and m are the slopes of the reference and compound respectively, η is the refractive index of the medium used. Here, we have investigated the QY of our probe (Fig.S5) by using quinine sulphate as standard reference which has an emission maxima at ~ 450 nm. And also, fluorescence QY of quinine sulphate in 0.1M H_2SO_4 is 0.54. To measure QY of MPD

5MI at an excitation wavelength of 400 nm in DMF solution we adjust its absorbance to below 0.1 and then emission spectrum was monitored. By using Eqn 1, we found that the QY of our probe is of 90%.

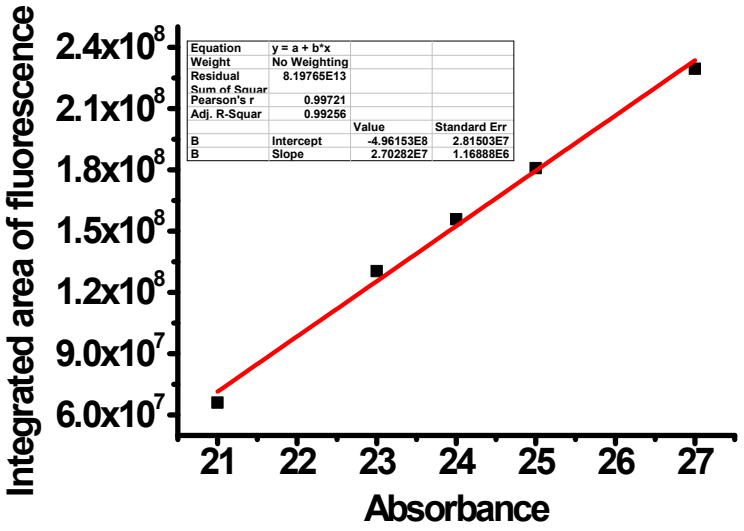


Fig. S5. A plot of absorbance vs. Integrated area of fluorescence determining the QY of MPD 5MI.

S7. Cyclic Voltammetry studies

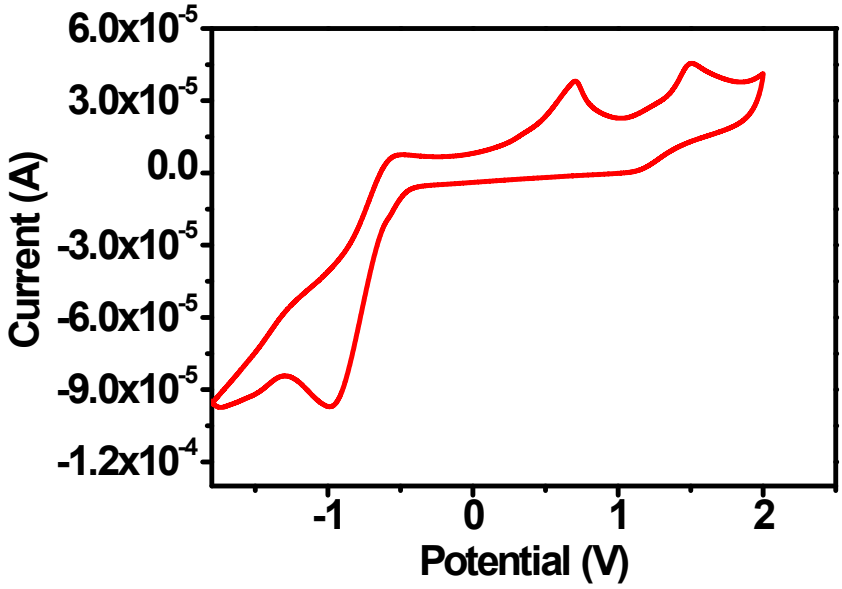
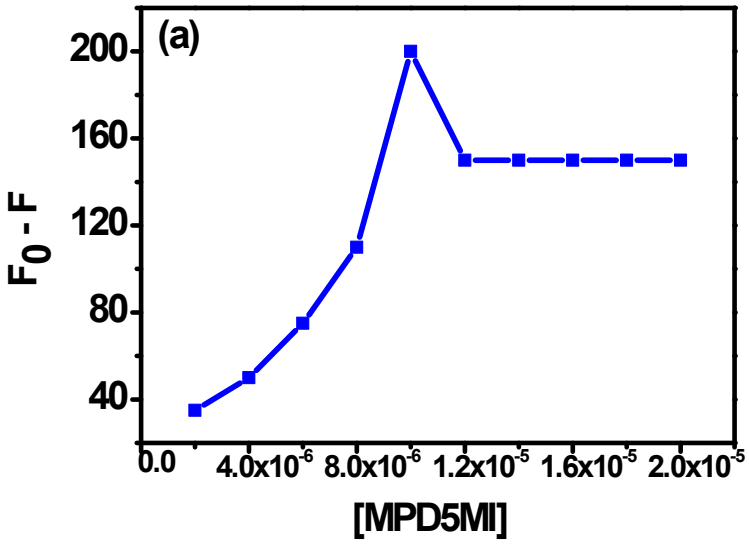
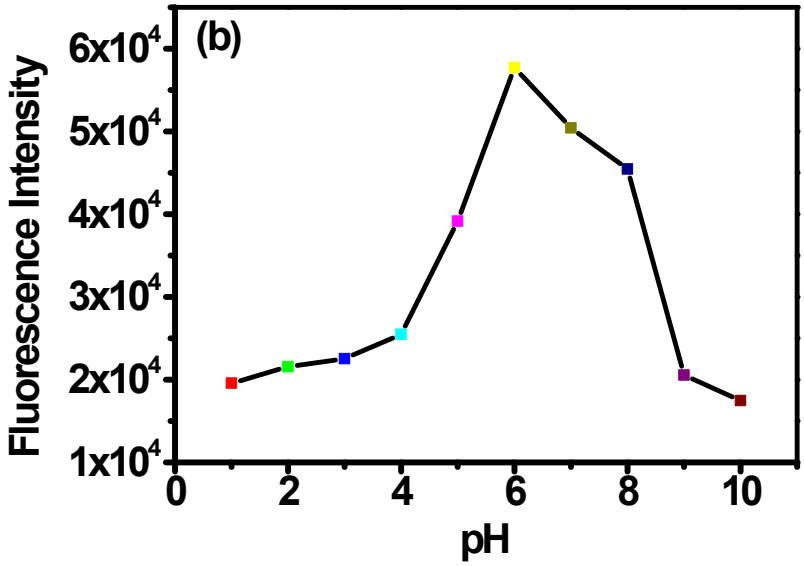


Fig.S6. Cyclic voltammogram responses of MPD 5MI

S8. Optimization parameters





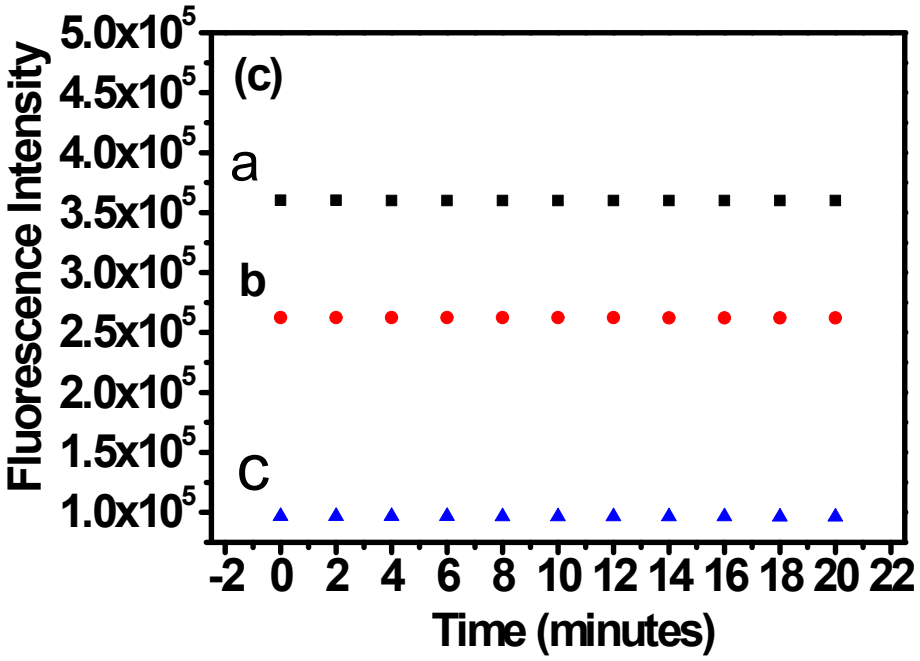


Fig. S7. (a) Effect of MPD 5MI (1 x10^{-6} M to 2x10^{-5} M) on the fluorescence change of the system. (b) Effect of pH (1-10) on the MPD 5MI (c) Effect of time on the fluorescence intensity of a Cys, b. Cys + M MPD 5MI (1 × 10^{-5} M), and c Cys+MPD 5MI (10 × 10^{-5} M)

S9. Limit of Detection

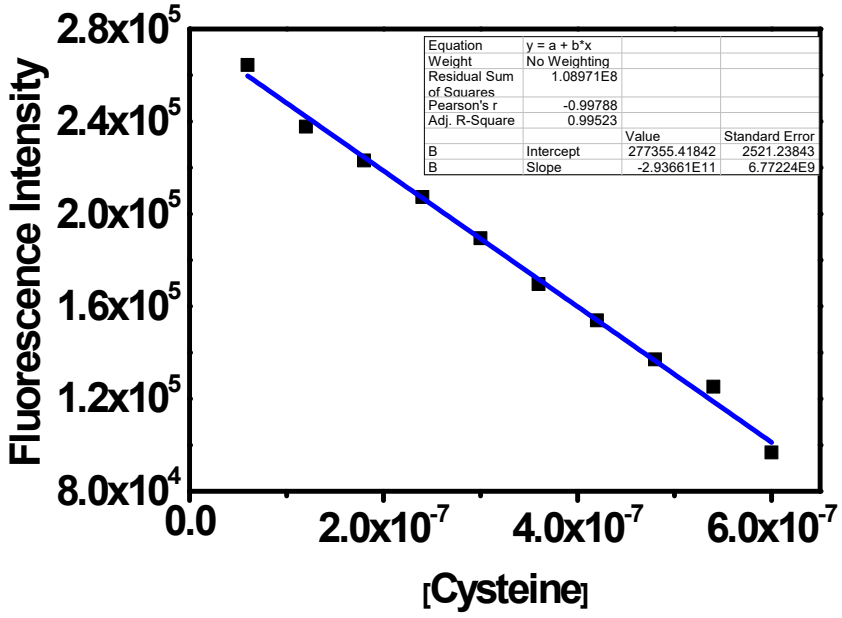


Fig.S8. A linear calibration curve for calculating the LOD (Inset shows R^2value = 0.9952)

S10. Stern Volmer plot

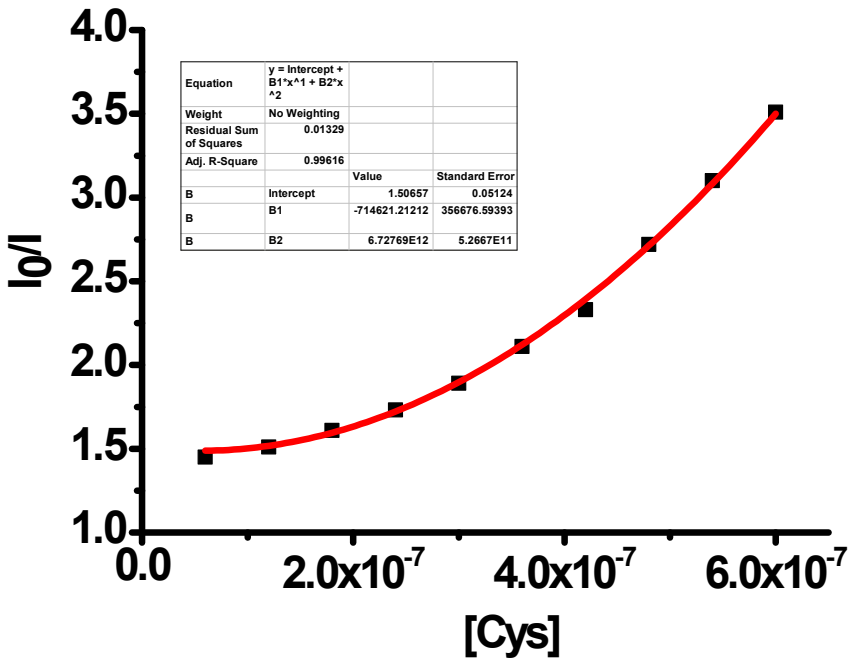


Fig.S9. Stern Volmer Plot of MPD 5MI with Cys

S11. Lifetime spectrum of probe with and without analyte

Determination of fluorescence lifetime of MPD 5MI: Lifetime measurements are also important as it is the time available for the fluorophore to interact with the environment. We have done the steady state lifetime measurements to study the excited state dynamics of the probe . A high value of average lifetime (7.32 ns) to the probe obtained after the measurements. Tri-potential fitting was applied for measuring the lifetime with minimum reduced chi value of 1.19. the τ, τ_{avg} obtained for MPD 5MI was summarized in Table S5.

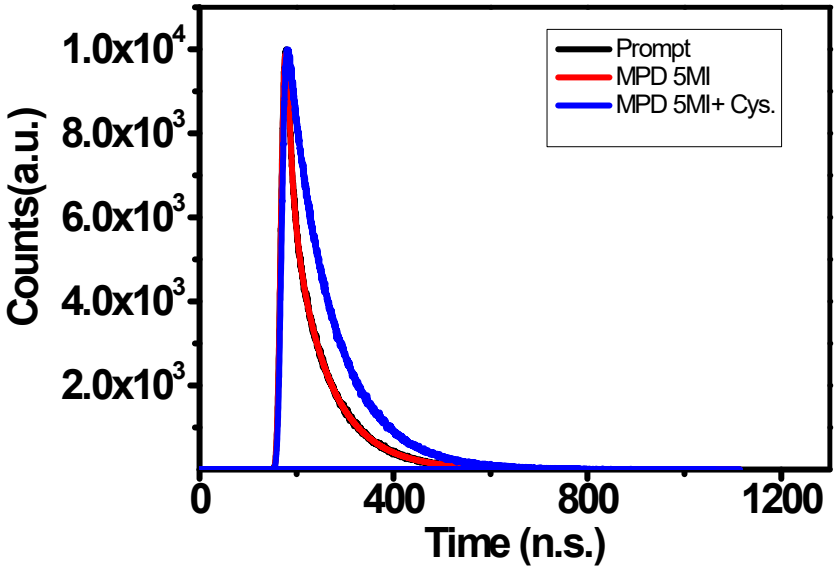


Fig. S10.Time resolved emission spectrum of 1 x 10^{-5} M MPD 5MI with and without the addition of 5 X 10^{-6} M Cys at pH 6 by exciting at 340 nm.

S12. Stern Volmer plot in terms of lifetime

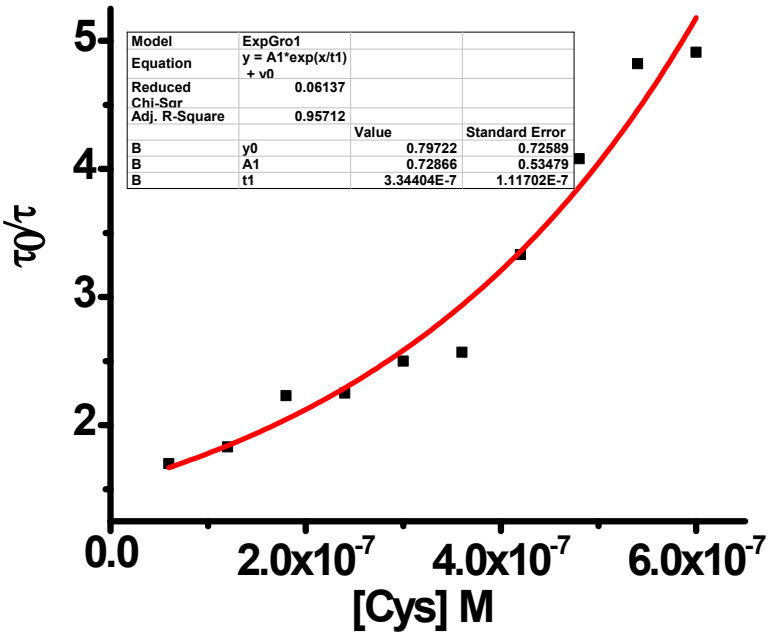


Fig. S11. Stern Volmer Plot of MPD 5MI with Cys in terms of fluorescence lifetime measurements.

S13. Overlap Spectra

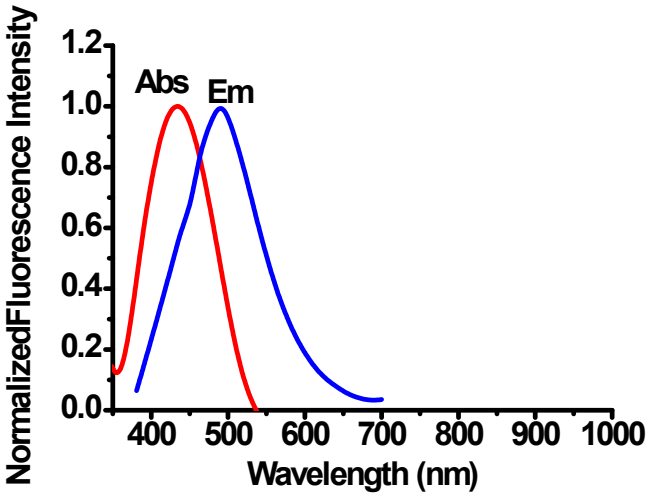


Fig.S12. Spectral overlap of emission spectrum of Cys (blue) and absorption spectrum of MPD 5MI (red).

Table S1.

Electrochemical characteristics of the probe

Probe	**Ep**	**Eh**	**Ip**	**Ah**	**Band gap**
MPD 5MI	-1.89V	-0.21V	$4.09e^{-6}$ A	$5.38e^{-6}$C	2.21eV

Where, Ep is the the cathodic potential corresponding to the cathodic current I_p. Eh is the anodic peak potential corresponding to the anodic current Ah.

Table. S2.

Electronegativity (χ) = $\frac{I + A}{2}$ Eqn 2

Chemical hardness (η) = $\frac{I - A}{2}$ Eqn 3

Chemical potential (Pi) = $-\chi$ Eqn 4

Chemical softness (S) = $\frac{1}{2\eta}$ Eqn 5

Electrophilicity index (ω) = $\frac{\mu^2}{2\eta}$ Eqn 6

Where I is the ionization potential given by $-E_{HOMO}$ and A is the electron affinity obtained from $-E_{LUMO}$ [51].

The calculated quantum chemical parameters of the probe.

Probe	**$-E_{HOMO}$ (eV)**	**$-E_{LUMO}$ (eV)**	**ΔE (eV)**	**χ (eV)**	**η(eV)**	**Pi (eV)**	**S (eV)**	**ω (eV)**	**Dipole moment (Debye)**
MPD 5MI	5.07	2.14	2.93	3.61	1.47	-3.61	0.34	3.63	3.27

Table.S3.

Comparative study on the determination of Cys with other reported sensors

Material	**Linear range (μM)**	**Limit of Detection (μM)**	**Reference**
N'1,N'4-bis((E)-2-hydroxy-3,5-diiodobenzylidene)terephthalohydrazide	0-200	0.055	[55]
4,4-Difluoro-4-bora-3a,4a-diaza-s-indacene	0-500	0.118	[56]
integration of fluorescein and coumarin fluorophores	0-10	0.084	[57]
cyanine-based fluorescence probe	0-25	0.16	[58]
cinamaldehyde–pyrimidine-Hg^{2+} complex	0.1-5	0.10	[59]
MPD 5MI	0.2-0.5	3.97×10^{-5}	Present work

Table S4.

Fluorescence lifetime values of MPD 5MI with and without Cys

	Average lifetime (sec)	τ values (sec)(α value in%)
MPD 5MI	7.32×10^{-9}	$\tau_1 = 4.34 \times 10^{-9}$ s (1) $\tau_2 = 8.85 \times 10^{-9}$ s (81) $\tau_3 = 8.59 \times 10^{-10} s$ (19)
MPD 5MI + Cys	4.24×10^{-9}	$\tau_1 = 3.01 \times 10^{-9}$ s (13) $\tau_2 = 7.00 \times 10^{-9}$ s (53) $\tau_3 = 4.40 \times 10^{-10} s$ (34)

Table S5

Parameters for multi-exponential fit of emission decay traces for MPD 5MI and MPD 5MI/Cys systems by exciting the samples at 340 nm.

Sample	**Fluorescence Lifetime (ns)**			**Average Lifetime (ns)**	**τ_0/τ**
	τ_1	**τ_2**	**τ_3**	**τ_{av}**	
MPD 5MI (τ_0)	4.34 (1%)	8.85 (81%)	8.59 (19 %)	7.32	1
MPD 5MI/Cys (τ)	3.01 (13 %)	7.00 (53 %)	4.40 (34 %)	4.24	1.70
	3.02 (16 %)	8.07 (30 %)	6.87 (62 %)	3.94	1.83
	2.95 (8 %)	8.53 (2 %)	62.27 (86 %)	3.24	2.23
	2.39 (12 %)	7.92 (32 %)	6.31 (56 %)	3.20	2.25

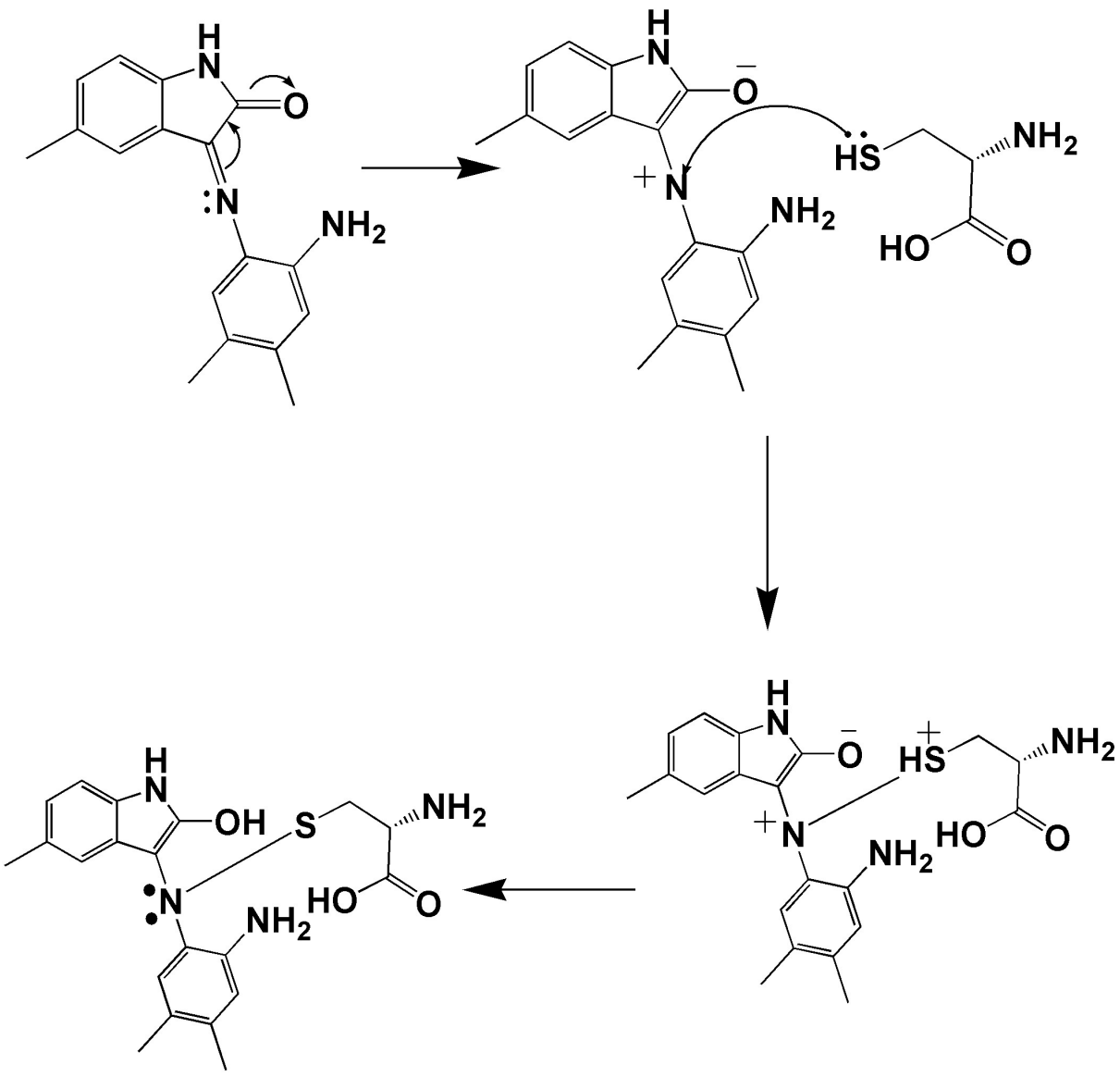
	2.18 (12 %)	7.71 (29 %)	6.12 (59 %)	2.89	2.50
	2.67 (11%)	7.98 (26 %)	6.75 (63 %)	2.81	2.57
	3.50 (20 %)	7.13 (66 %)	7.20 (14 %)	2.17	3.33
	3.80 (30 %)	7.27 (69 %)	17.1 (1 %)	1.77	4.08
	7.72 (71 %)	3.03 (28 %)	17.3 (1 %)	1.50	4.82
	7.56 (71 %)	2.66 (27 %)	10.5 (2 %)	1.47	4.91

Table. S6.

Determination of Cys in real samples where n= 5

Sample	Added (M)	Found (M)	Recovery (%)	RSD (%)
Synthetic urine	1×10^{-6} to 4×10^{-7}	0.98×10^{-6} to 3.88×10^{-7}	99.60	2.08
Blood serum	2×10^{-6} to 3×10^{-7}	2.12×10^{-6} to 3.12×10^{-7}	101.40	2.76

Scheme 2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N
O
:N
NH2
H
N
O
HS
NH2
N
NH2
HO
O
H
N
O
HS
NH2
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N
OH
S
NH2
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