

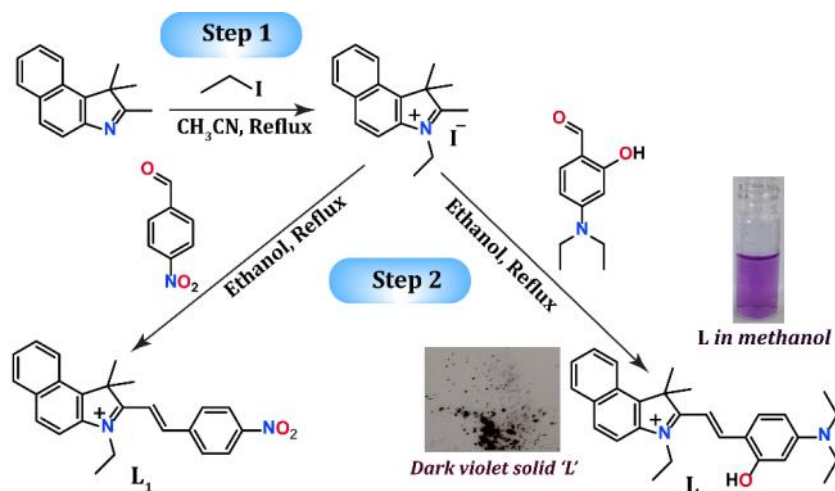
Supporting Information

A TICT Based TURN-ON Fluorogenic Nano-Probe for Realtime Detection of Serum Albumin in Physiological Condition

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Scheme S1: Synthesis of the probe **L** and **L₁**.

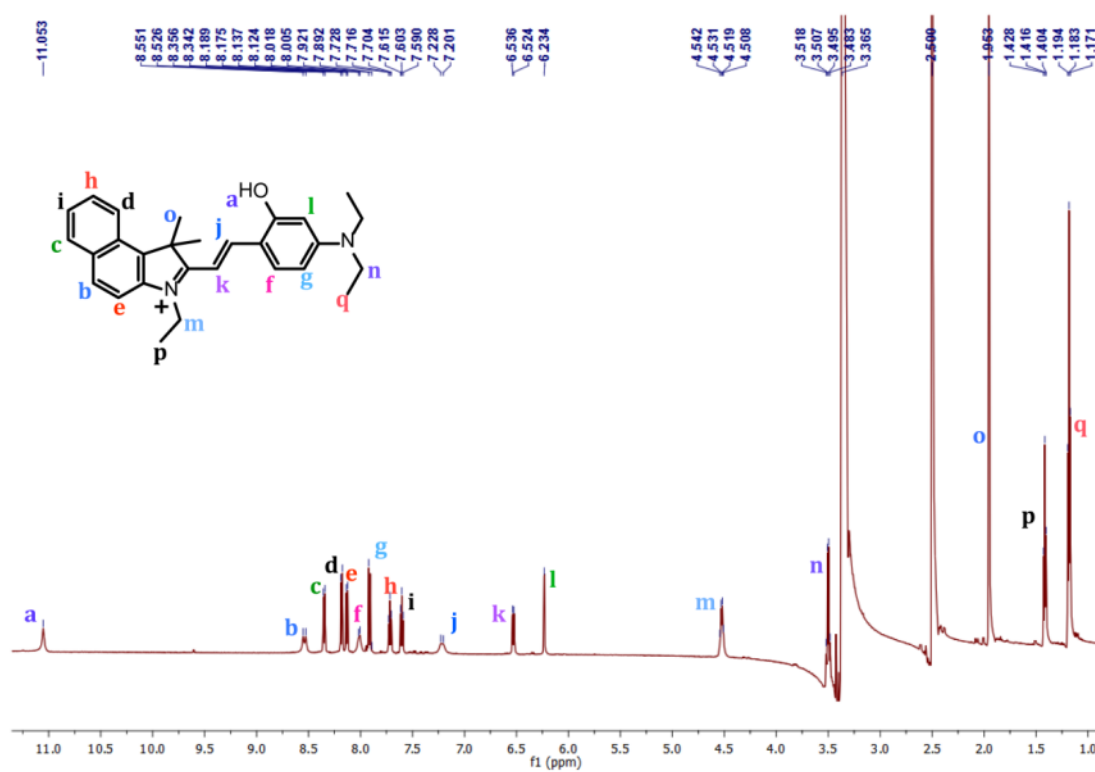


Figure S1: ¹H-NMR spectra of **L** in DMSO-d₆.

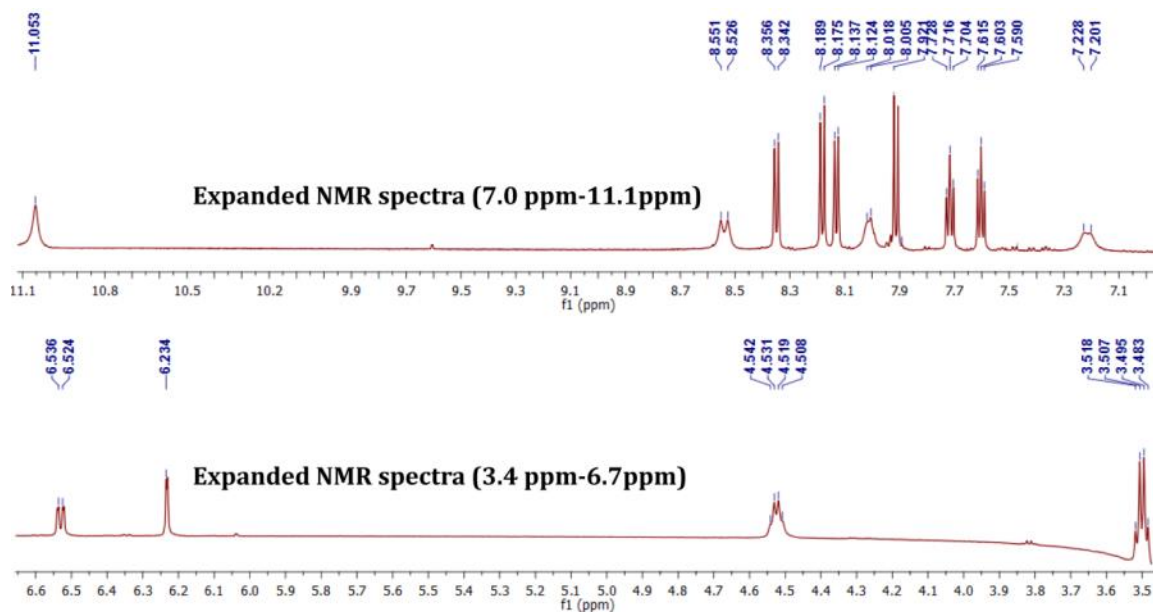


Figure S2: Expanded (3.4 ppm-11.1 ppm) ^1H -NMR spectra of **L** in DMSO-d_6 .

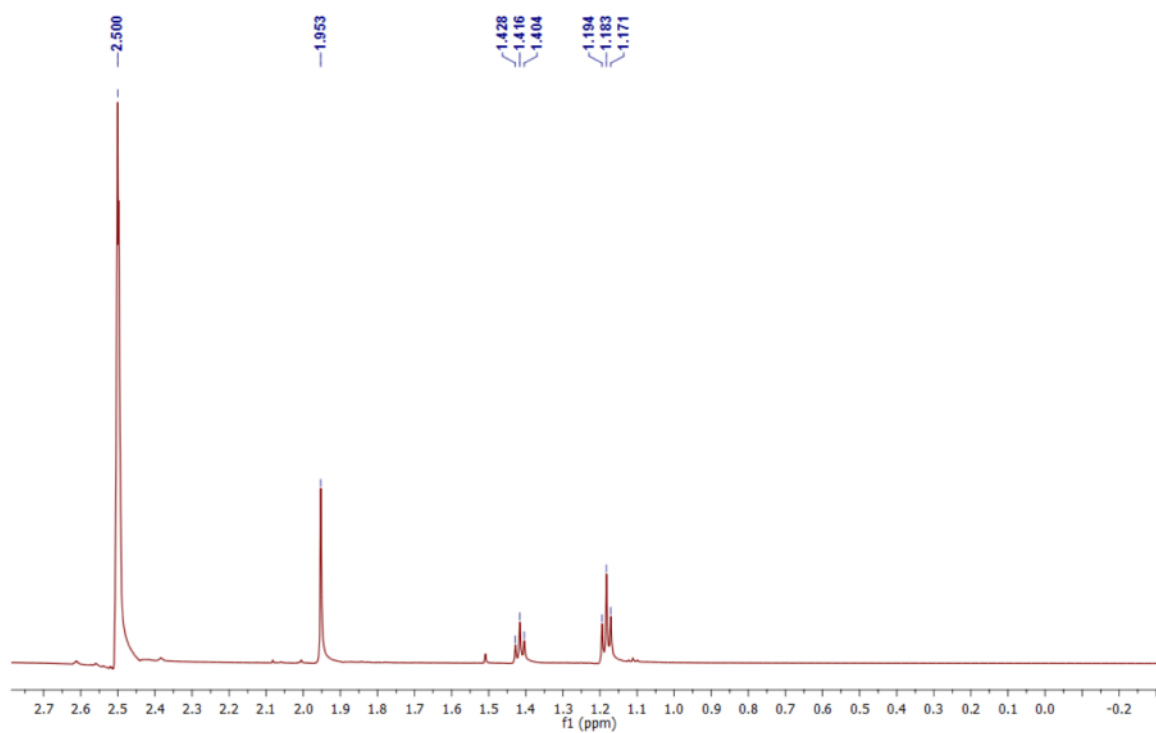


Figure S3: Expanded (aliphatic region) ^1H -NMR spectra of **L** in DMSO-d_6 .

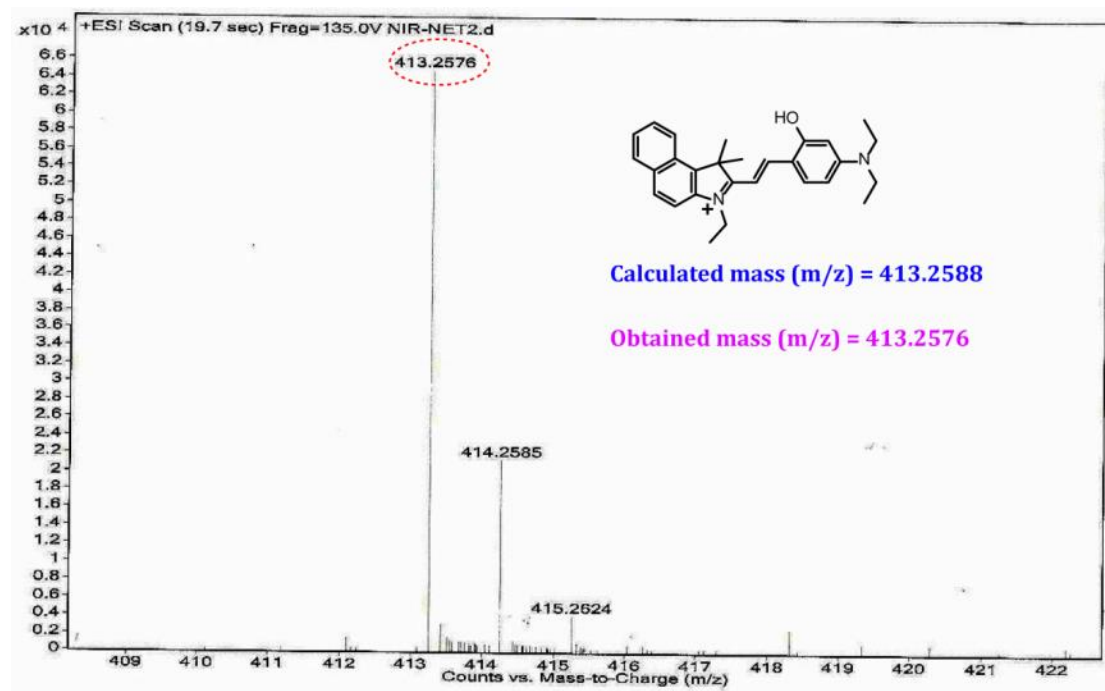


Figure S4: Mass spectrum of L.

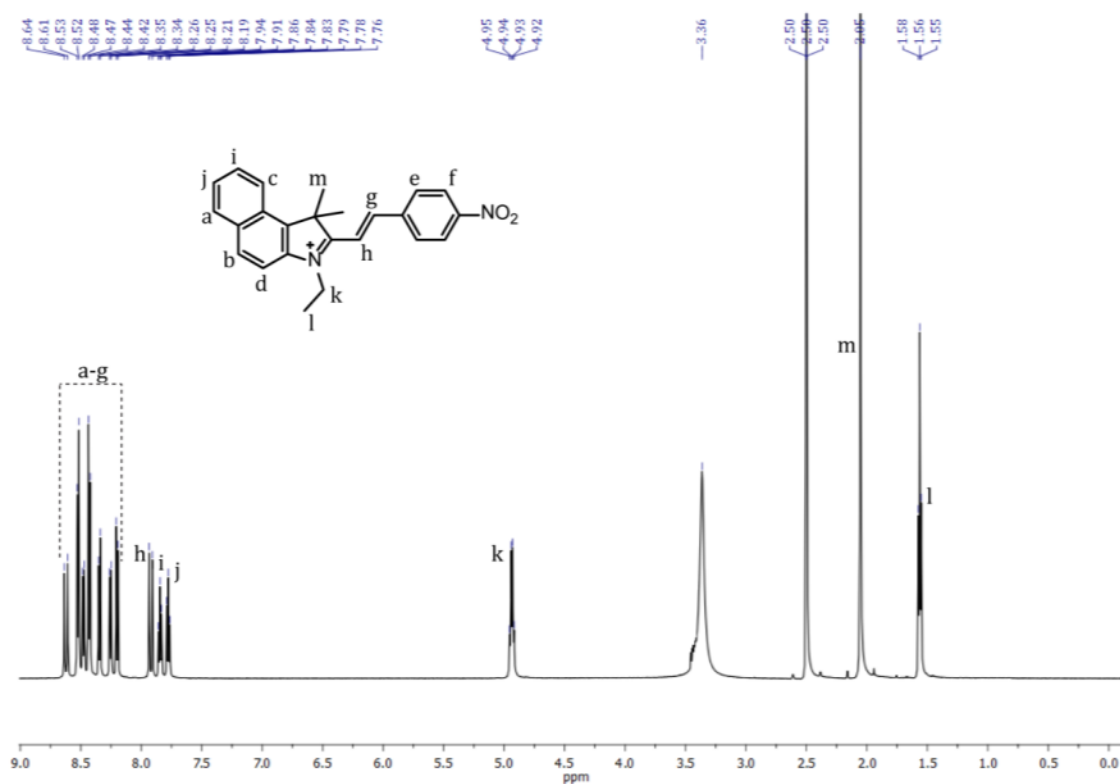


Figure S5: ¹H-NMR spectra of L₁ in DMSO-d₆.

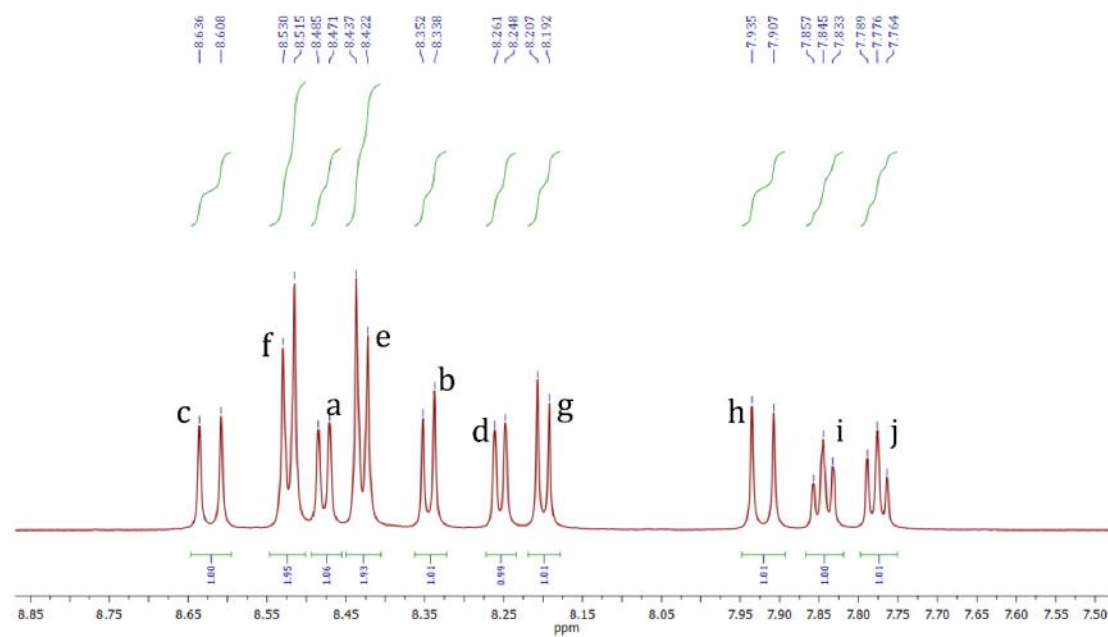


Figure S6: Expanded (aromatic region) ^1H -NMR spectra of L_1 in DMSO-d_6 .

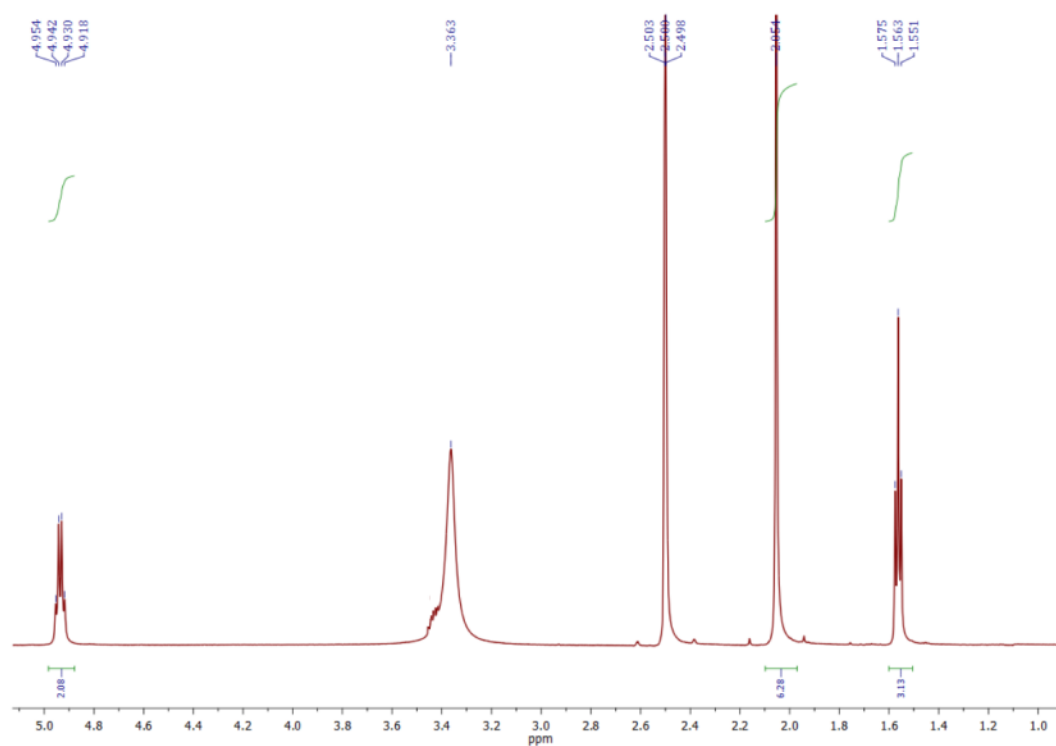


Figure S7: Expanded (aliphatic region) ^1H -NMR spectra of L_1 in DMSO-d_6 .

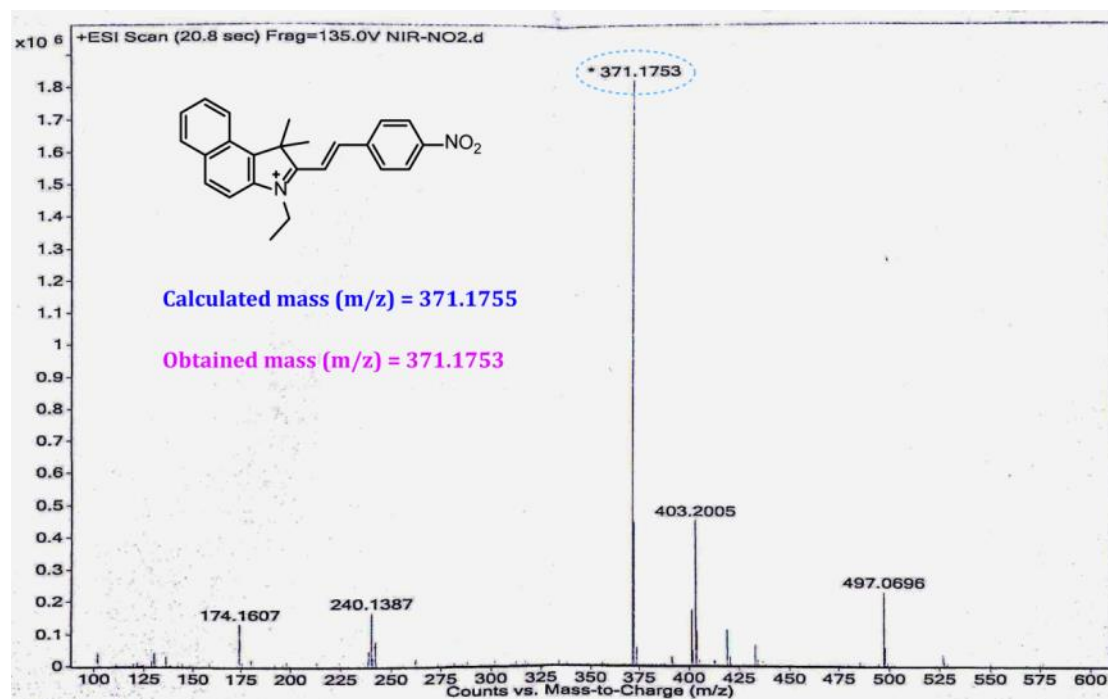


Figure S8: Mass spectrum of **L₁**.

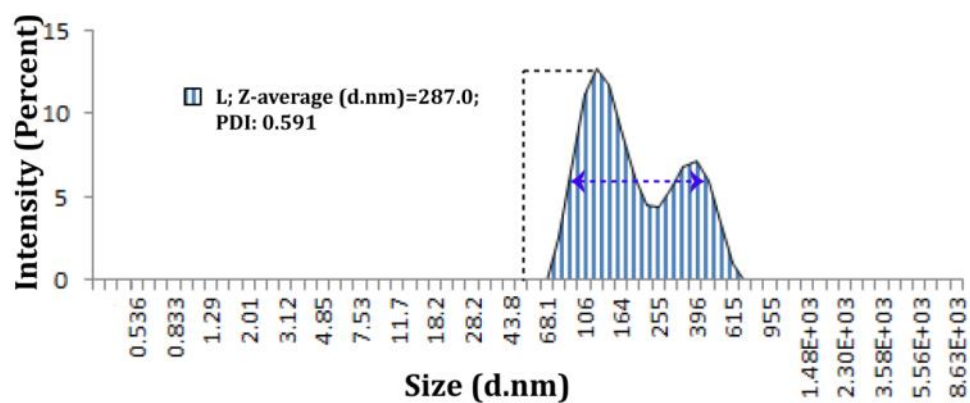


Figure S9: DLS-based particle size analysis of **L** (2 μ M) in aqueous phosphate buffer saline (PBS) solution (pH 7.4).

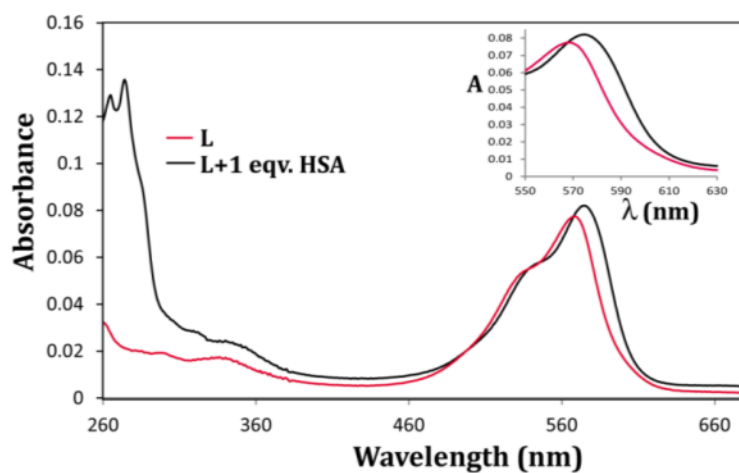


Figure S10: UV-visible spectra of **L** (2 μM) in $\sim 100\%$ aqueous PBS buffer (pH 7.4) medium in presence of 2.0 equivalents of HSA. INSET: Expanded portion of the spectra in the wavelength region 550 nm - 630 nm.

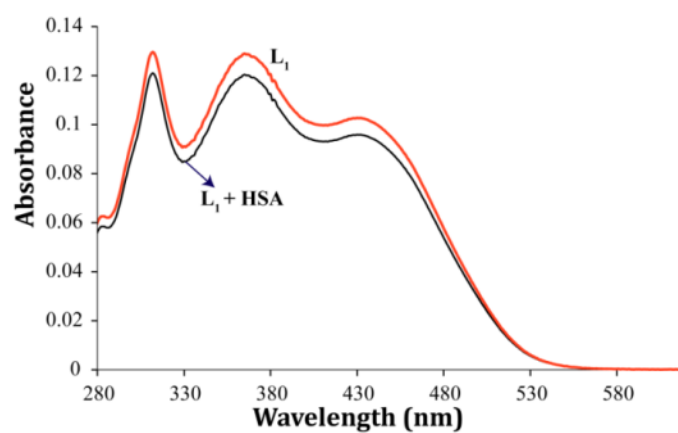


Figure S11: UV-visible spectra of **L₁** (2 μM) in $\sim 100\%$ aqueous PBS buffer (pH 7.4) medium in presence of 2.0 equivalents of HSA.

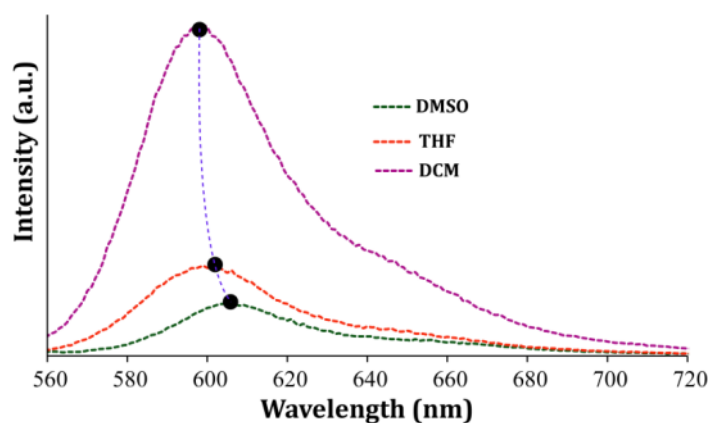


Figure S12: Fluorescence spectra of **L** (2 μM) in different solvents with varying solvent polarity.

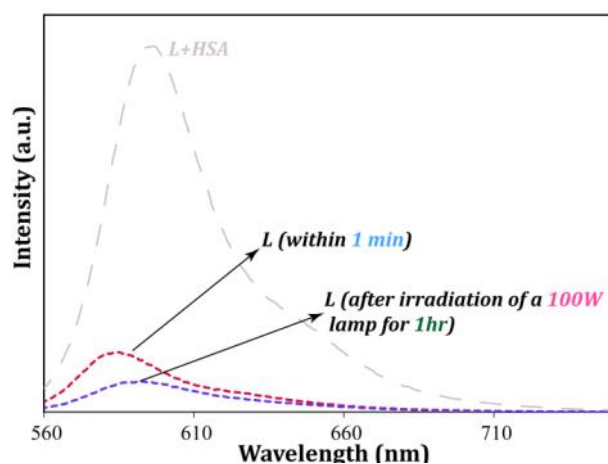


Figure S13: Fluorescence spectra of **L** (2 μ M) before and after the irradiation of a 100W lamp to the solution of **L** for 1 hr.

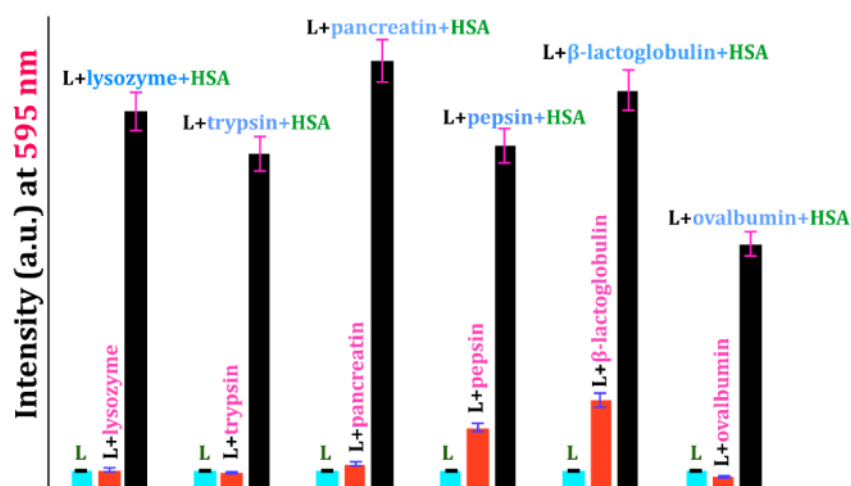


Figure S14: Changes in the emission intensity of (**L**+1 equivalent of HSA) at 595 nm in presence of various other proteins ; λ_{ex} =540 nm.

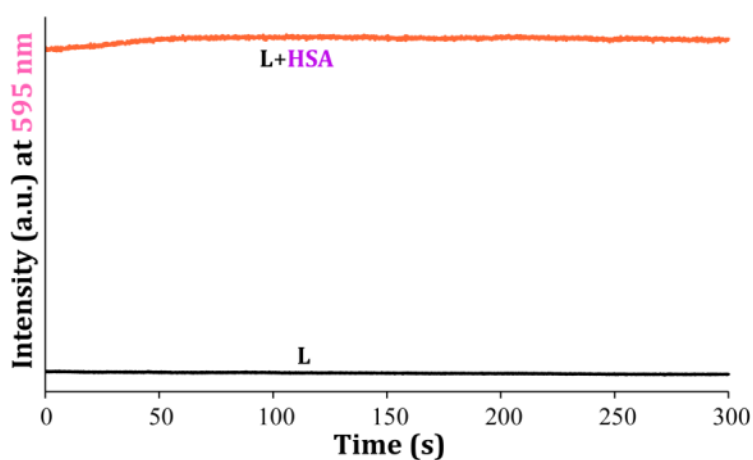


Figure S15: Changes in the emission intensity of **L** at 595 nm with time upon interaction with HSA; λ_{ex} =540 nm.

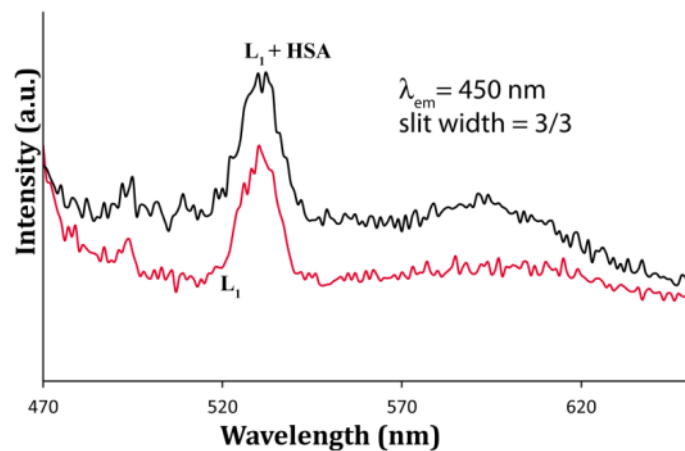


Figure S16: Fluorescence spectra of L_1 in presence of 2.0 equivalents of HSA in ~100% aqueous buffer (PBS, pH 7.4); λ_{ex} =450 nm.

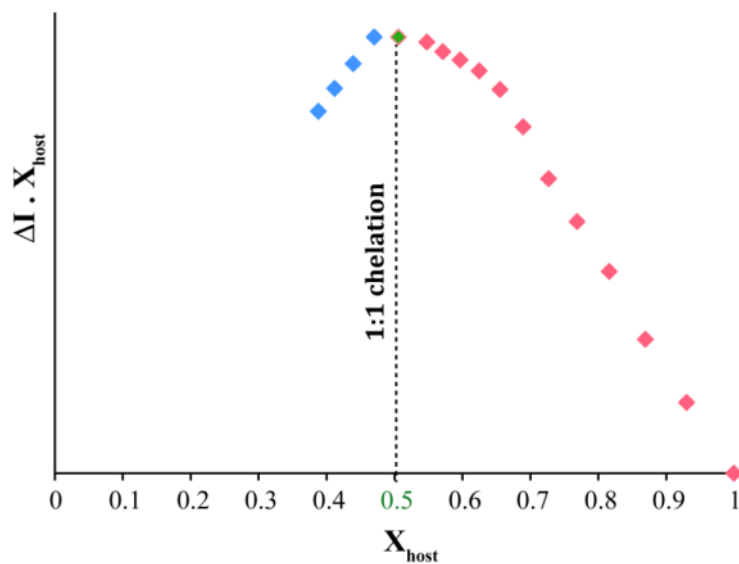


Figure S17: Job's plot obtained from the fluorescence titration experiment showing 1:1 chelation between L and HSA.

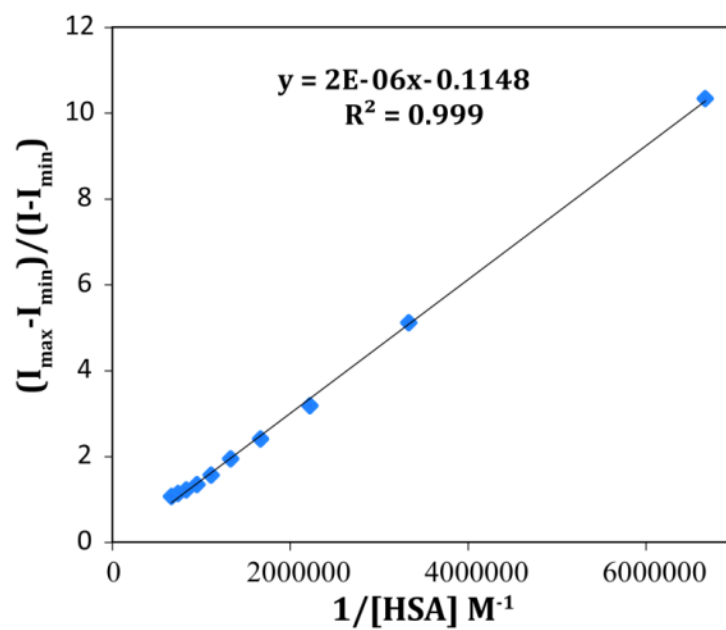


Figure S18: B-H plot for determination of binding constant (L-HSA complex).

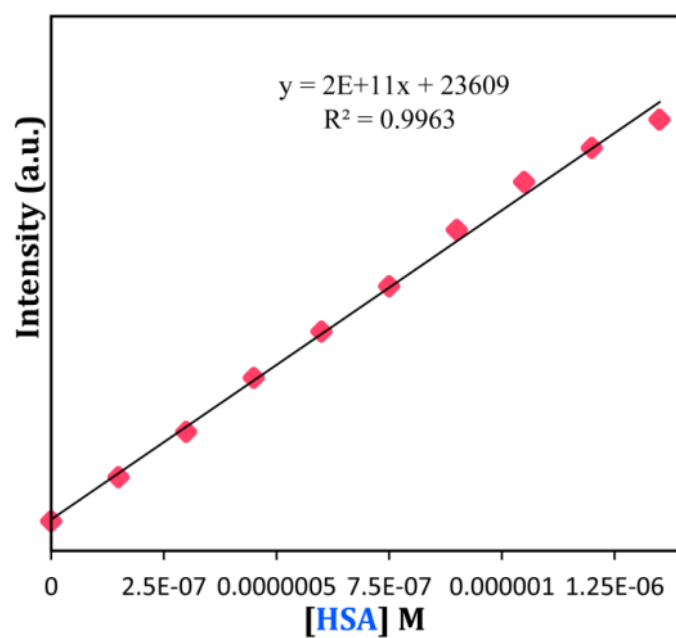


Figure S19: Fluorescence intensity (at 595 nm) vs. concentration of HSA plot for determination of detection limit.

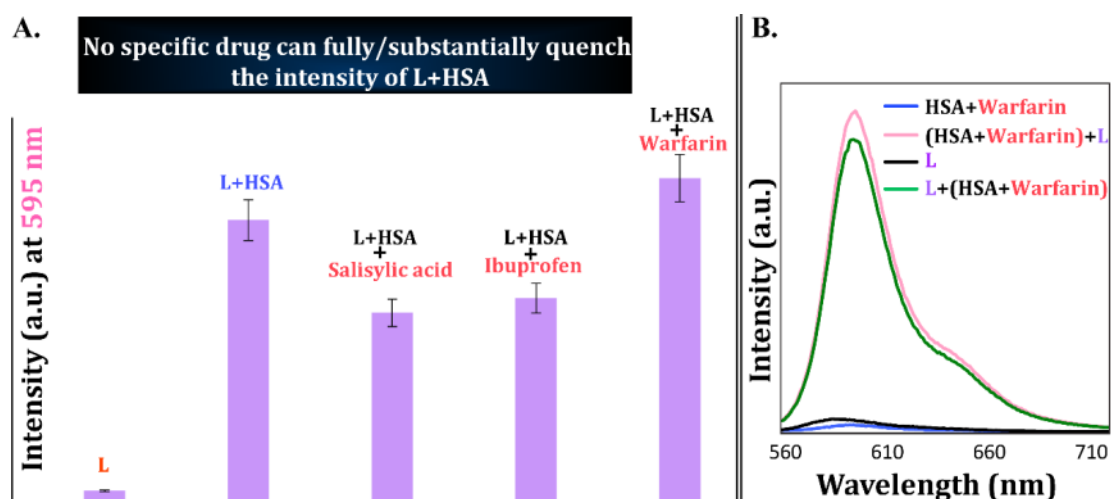


Figure S20: (A) Changes in the emission intensity of (L+1 equivalent of HSA) at 595 nm upon addition of various site specific drugs; (B) Reverse drug displacement study; $\lambda_{\text{ex}}=540$ nm.

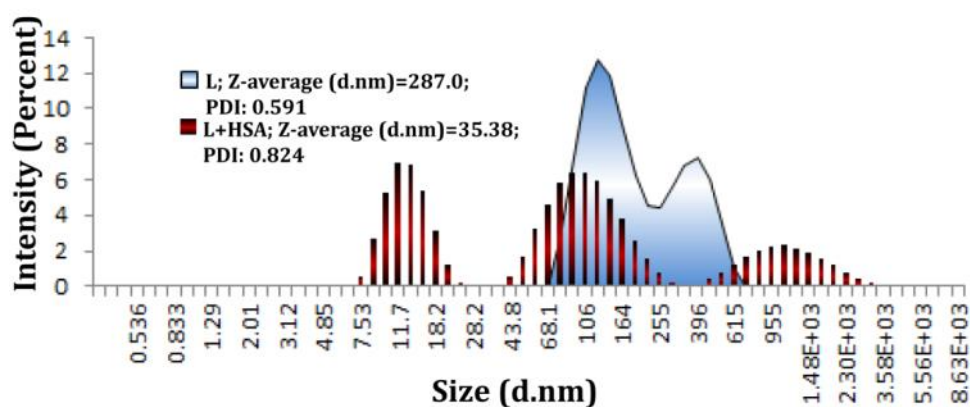


Figure S21: DLS-based particle size analysis; change in particle size of L (2 μM) in aqueous phosphate buffer saline (PBS) solution (pH 7.4) upon interaction with 1 equivalent of HSA.

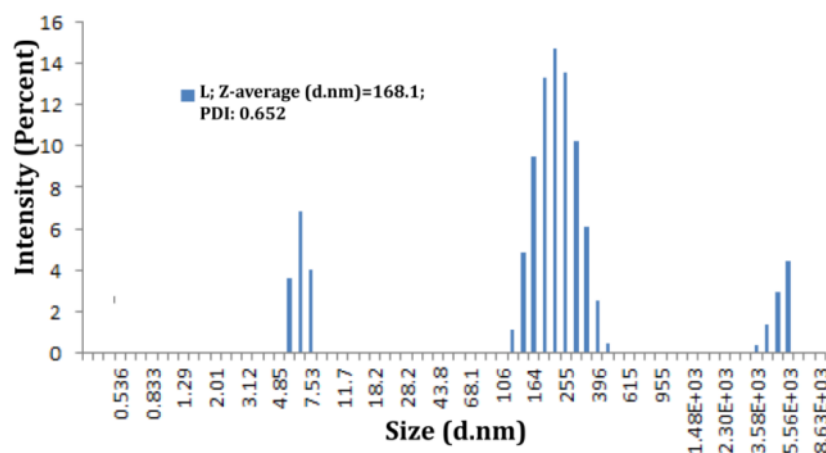


Figure S22: DLS-based particle size of L (2 μM) in aqueous phosphate buffer saline (PBS) solution (pH 7.4) upon interaction with SDS.

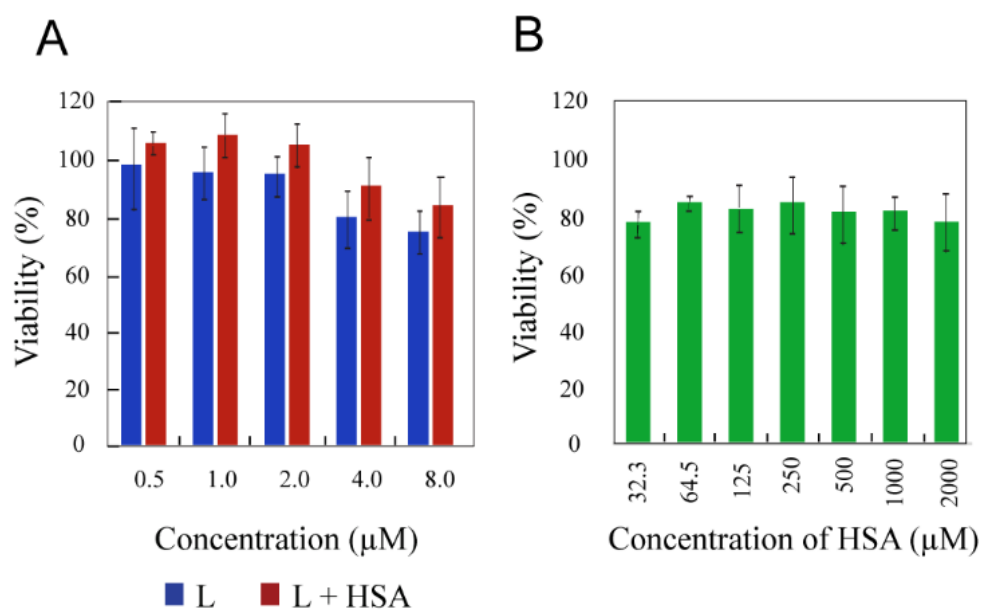


Figure S23: MTT based cytotoxicity assay for (A) probe **L** , **L**/HSA ensemble and (B) only HSA.

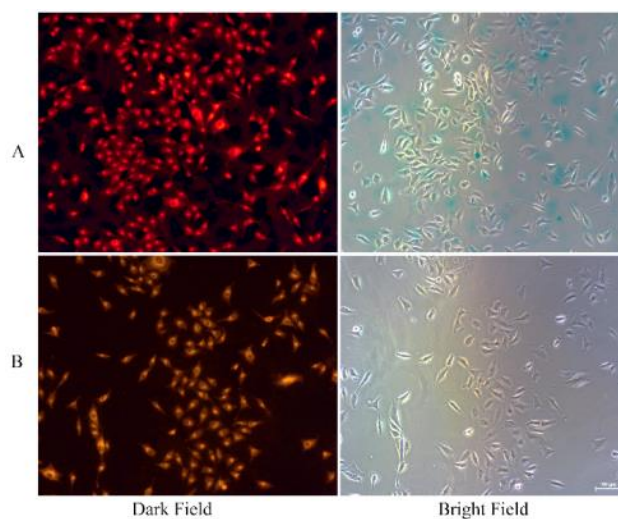


Figure S24: Fluorescence microscope images of HeLa cells incubated with (A) probe **L** (2 μM) only (B) probe **L** (subsequent addition of 125 $\mu\text{g/ml}$ HSA)

Hydrophobic Interactions ****

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	191B	ALA	3.57	11276	7465
2	194B	ALA	3.55	11278	7487
3	195B	LYS	3.87	11273	7494
4	195B	LYS	3.79	11279	7493
5	198B	LEU	3.57	11271	7538
6	214B	TRP	3.77	11264	7700
7	343B	VAL	3.18	11257	8936
8	447B	PRO	3.10	11263	9954
9	451B	ASP	3.66	11264	9984
10	451B	ASP	3.62	11260	9984
11	455B	VAL	3.13	11271	10026
12	455B	VAL	3.05	11279	10025
13	456B	VAL	3.49	11286	10034

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Sidechain	Donor Atom	Acceptor Atom
1	436B	LYS	1.97	2.76	133.88	✓	✓	9843 [N3+]	11280 [O3]

Figure S25: Interactions encountered in the Docking conformation of HSA/L ensemble with the lowest binding free energy obtained from Protein-Ligand Interaction Profiler.

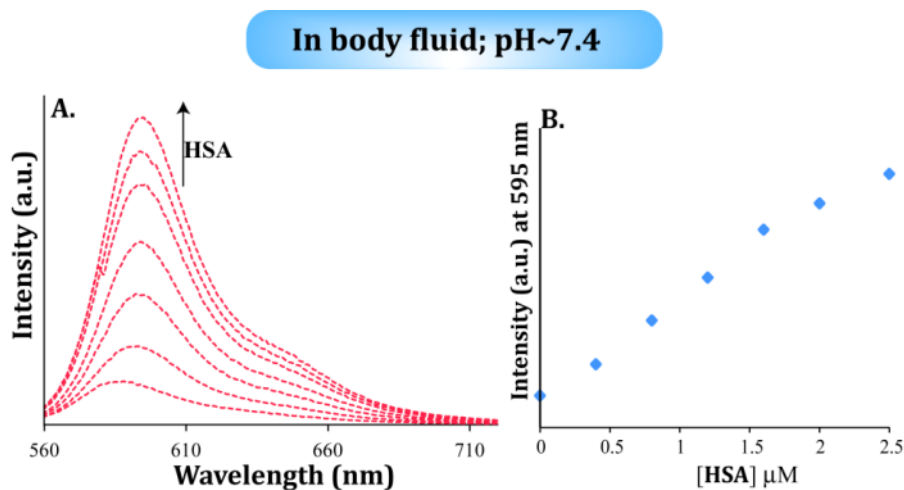


Figure S26. (A) Fluorescence spectra of **L** (2 μM) in presence of varying concentration of HSA in body fluid; pH~7.4; (B) Changes in the emission intensity at 595 nm with concentration of HSA.

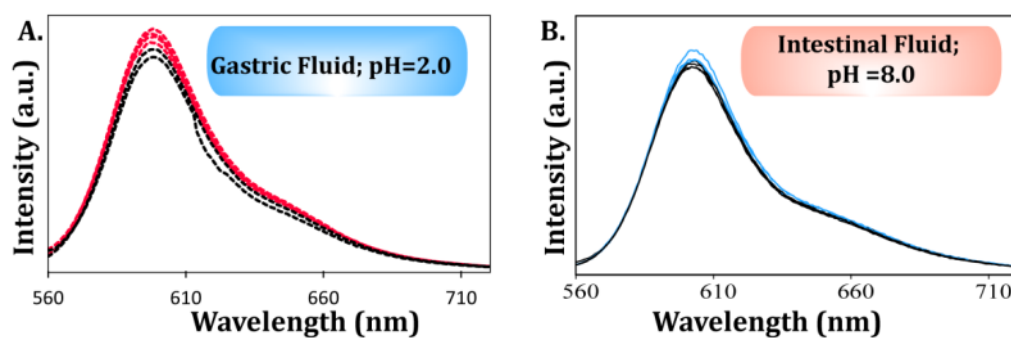


Figure S27: Fluorescence spectra of **L** (2 μ M) in presence of increasing concentration of HSA in (A) Gastric fluid; pH~2.0 and (B) Intestinal fluid; pH~8.0.

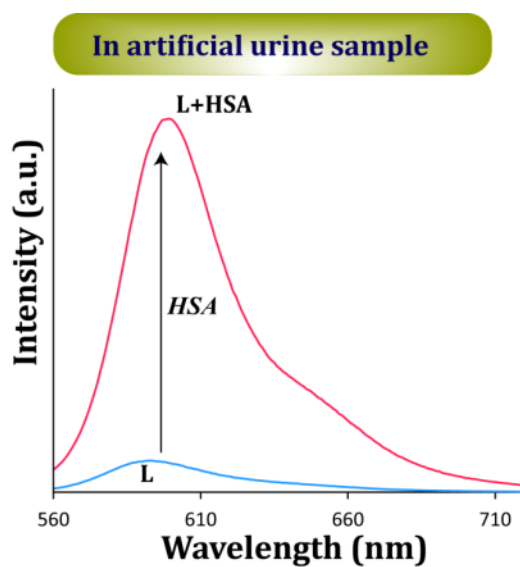


Figure S28: Fluorescence spectra of **L** (2 μ M) in presence of 1 equivalent of HSA in artificial urine sample