Supporting information

Red and NIR active dipod-SDS self-assemblies for "turn on" quantification of spermine in serum, urine and food; Smart-phone assisted on-site determination of spermine in amine rich foods

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1. Experimental

1.1 Materials and Instruments.

All chemicals were purchased from Spectro Chem, Sigma Aldrich or TCI chemicals and used as received. TLC was performed on aluminium sheets coated with silica gel 60F254 (Merk, Darmstadt). Deionized water was obtained from ULTRA UV/UF Rions Lab Water system Ultra 370 series. NMR spectra were recorded on Jeol 400 MHz NMR spectrometer with TMS as internal standard. Abbreviation's used for the splitting pattern are s = singlet, d = doublet, t = triplet, q = quartet and m = multiplet. Mass spectrum was obtained from mass Bruker micro TOF QII mass spectrometer. DLS measurements were performed at 25.0 $\pm$ 0.1°C using Zetasizer nano ZS instrument.

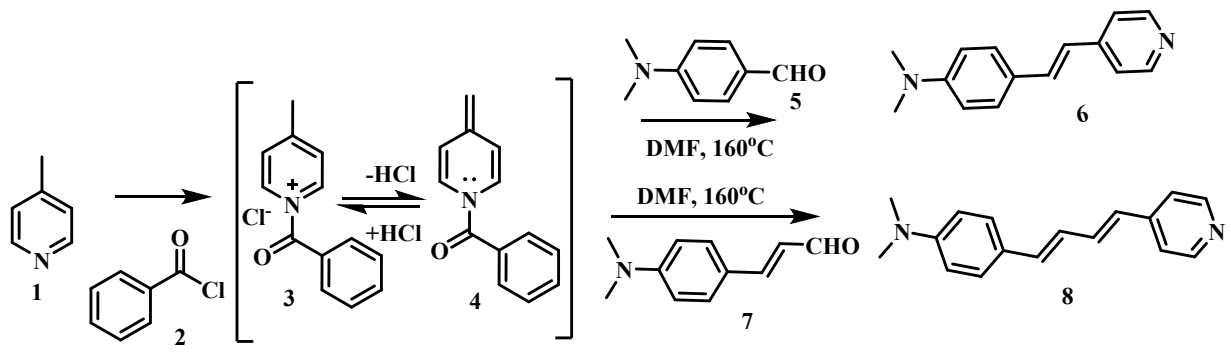
1.2. Synthesis

1.2.1 Synthesis of diene 6[1]

4-Methylpyridine (4 mmol, 388 µl) and benzoyl chloride (2 mmol, 232 µl) were dissolved in DMF (10 ml). The reaction mixture was stirred at room temperature for 30 min. Then N, N-dimethylbenzaldehyde (2 mmol, 298 mg) was added and reaction mixture was stirred at 160°C for 5 h. After completion of reaction, DMF was distilled under vacuum and the crude product was purified using column chromatography (SiO_2, EtOAc/ $CHCl_3$, 0.2:9.8 v/v) to get diene **6**, 360 mg, yield 80%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.83 (s, 6H, 2 x NCH_3), 6.52 (d, J = 8.7 Hz, 2H, 2 x ArH), 6.61 (d, J = 16.2 Hz, 1H, alkene H), 7.06 (d, J = 16.2 Hz, 1H, alkene H), 7.14 (d, J = 4.8 Hz, 2H, 2 x ArH), 7.25 (d, J = 8.7 Hz, 2H, PyH), 8.31 (d, J = 5.1 Hz, PyH).

1.2.2 Synthesis of diene 8[2]

4-Methylpyridine (4 mmol, 388 µl) and benzoyl chloride (2 mmol, 232 µl) were dissolved in DMF (10 ml). The reaction mixture was stirred at room temperature for 30 min. Then N, N-dimethylcinnamaldehyde (2 mmol, 350 mg) was added and reaction mixture was stirred at 160°C for 5 h. After completion of reaction, DMF was distilled under vacuum and the crude product was purified using column chromatography (SiO_2, EtOAc/ $CHCl_3$, 0.2:9.8 v/v) to get diene **8,** 150 mg, 30% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.99 (s, 6H, 2 x CH_3), 6.46 (d, J =15.5 Hz, 1H, ArH), 6.69 (d, J = 8.5 Hz, 2H, ArH), 6.72-6.80 (m, 2H, ArH), 7.12 (dd, J_1 = 5.5 Hz, J_2 = 10.0 Hz, 1H, ArH), 7.24 – 7.25 (m, 3H, ArH), 7.36 (d, J = 8.5 Hz, 2H, ArH), 8.50 (d, J = 4.5 Hz, 2H, ArH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 40.3, 76.7, 77.0, 77.3, 112.3, 120.4, 123.9, 125.03, 126.9, 127.9, 134.5, 136.4, 150.0, 150.5.



Scheme S1: Synthesis of diene 6 and 8

References:

1. S. Dhiman, R. Kour, S. Kaur, P. Singh and S. Kumar. *Bioorganic Chem*., 2022, **129**, 106169.
2. S. Li, D. Song, W. Huang, Z. Li and Z. Liu, *Anal. Chem.* 2020, **92**, 2802−2808.

1.3 UV-Visible and fluorescence Studies

The UV-Vis spectra were recorded on SHIMADZU-2450 spectrometer equipped with a peltier system as the temperature controller set at 25.0 ± 0.1°C by using reference solvent in one of the quartz cuvette and sample in the second cuvette. The quartz cuvettes of 1 cm path length were used for recording the absorption spectrum. The fluorescence spectra were recorded on Fluorolog Horiba scientific model: FL-1039A/40A by using 1 cm path length quartz cuvette. At least 3 ml of the solution was added and stored for at least 2 minutes before recording the spectrum. Each spectrum was stored as ASCII file and then transferred to excel for processing the data. The solutions of R-SPM (10 μM), NIR-SPM (10 μM) and R-SPM∩SDS(10 μM∩500μM)**,** NIR-SPM∩SDS (10 μM∩500μM) for their respective titrations with SDS and spermine / spermidine, the aliquots of analyte (SDS/Spermine/Spermidine) were added and fluorescence spectra were recorded. Each experiment was performed three time. The standard deviation was determined for each addition.

1.4 Quantum Yield Calculation

The quantum yields (Φ) of solutions of **R-SPM and NIR-SPM** and their ensembles with SDS and SDS-spermine were measured using integrated sphere on FL-1039A/40A machine. The excitation wavelength was used as 490 nm.

1.5 DLS Sample preparation

The solvents DMSO and HEPES buffer were separately filtered through 0.02 μM filter to remove any suspended particles. The DLS samples were prepared by diluting the 30 μL of stock solution into either DMSO or deionized water or their mixture and were allowed to stand for 3 h so that solutions

become homogenous. For recording the DLS spectra, 1 ml of solution was taken into the glass cuvette of 1 cm path length and allowed to stand for 3 min before recording the spectra at 25°C. 5 measurements of each sample were recorded and mean of these records have been presented.

1.6 Detection limit

The detection limits of spermine and spermidine were determined as per IUPAC norms using the fluorescence data. For determining the S/N ratio, the emission spectra of the probes were recorded three times to find the standard deviation. The LOD was calculated by the equation LOD = 3σ/m where σ = standard deviation of blank measurements, m = slope of straight line of plot of emission intensity v/s concentration of analyte.

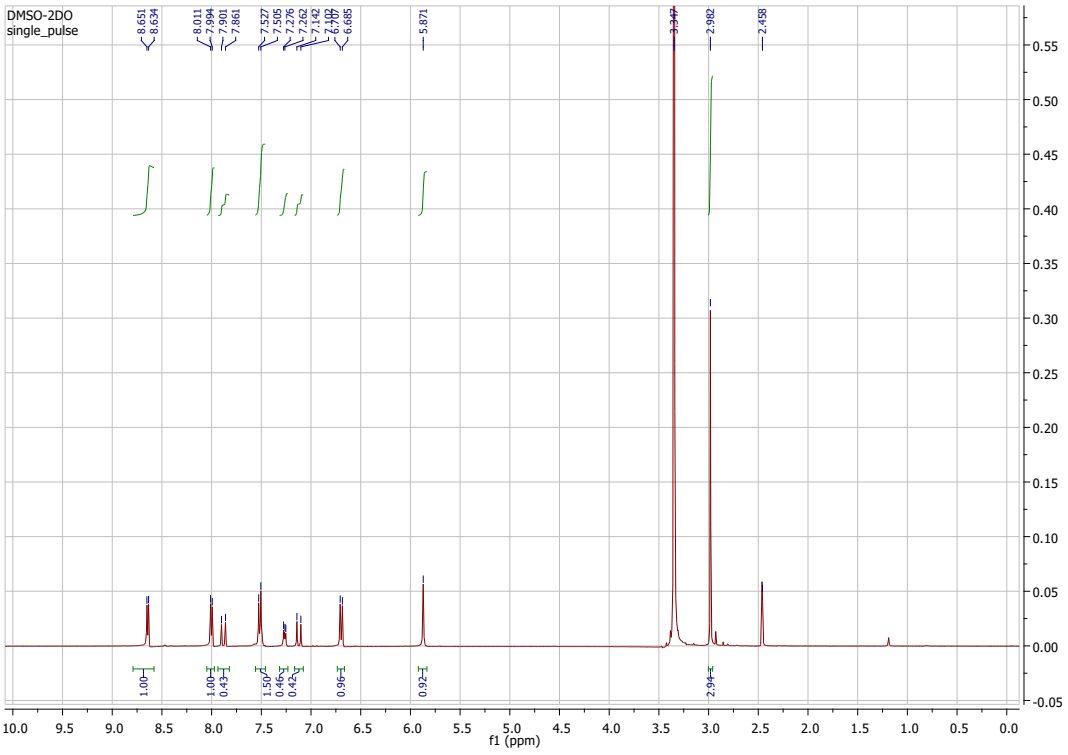


Figure S1. [1]HNMR spectrum of **R-SPM**

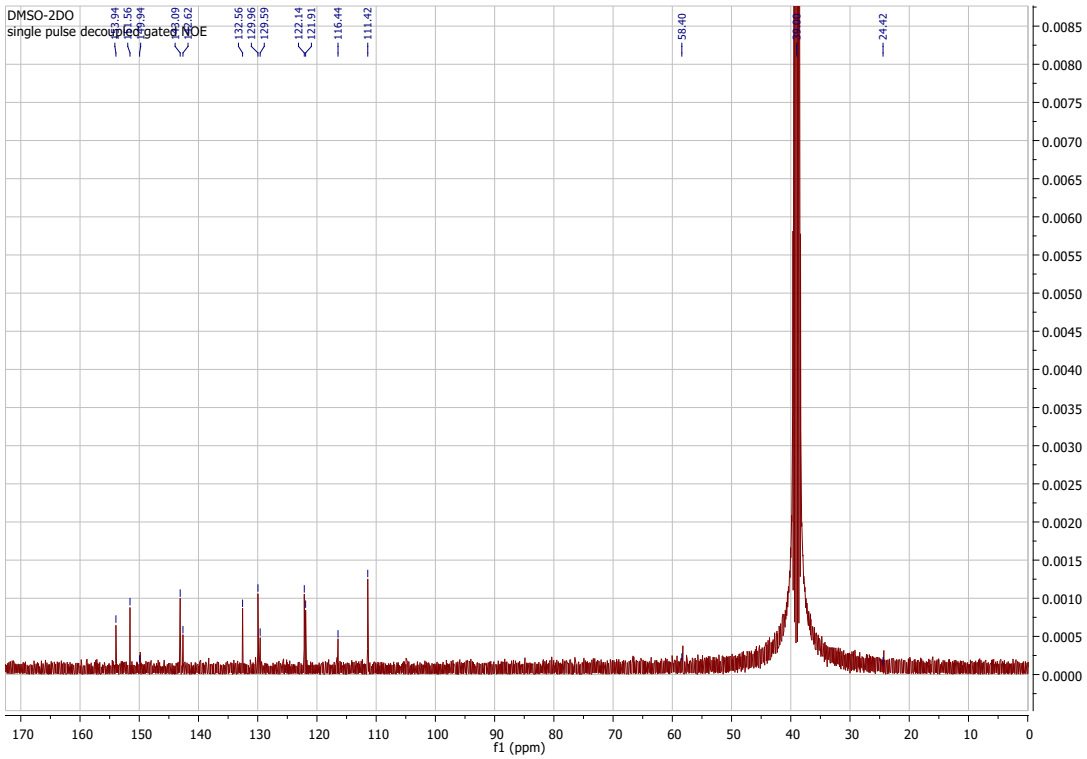


Figure S2. ^{13}CNMR spectrum of **R-SPM**

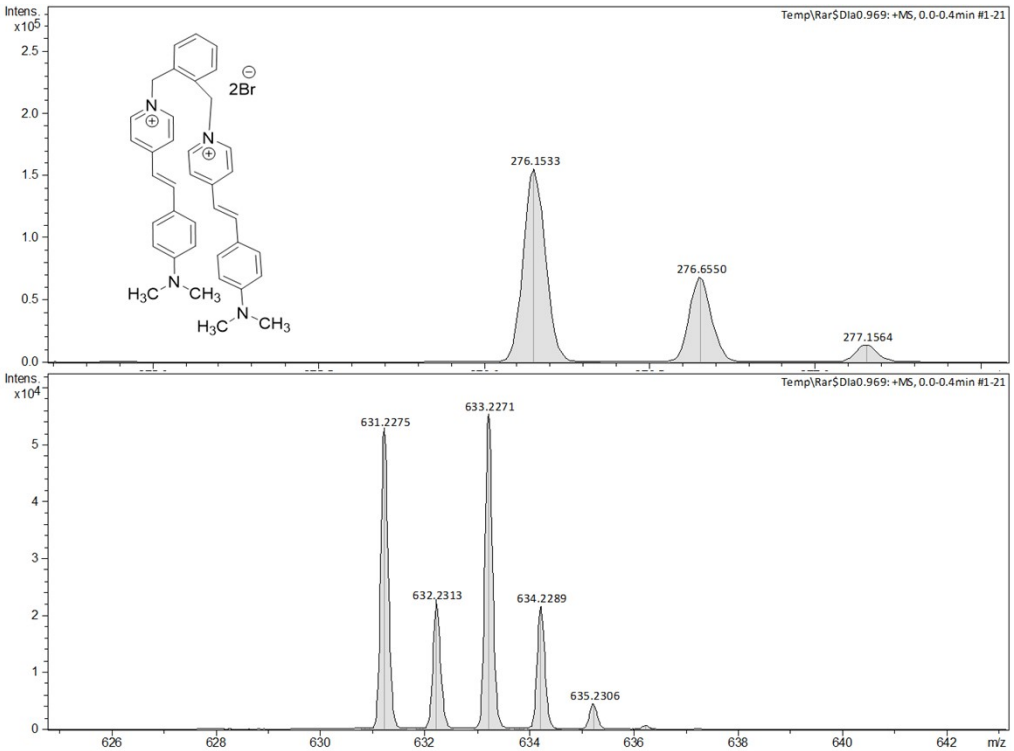


Figure S3: HRMS spectrum of **R-SPM**

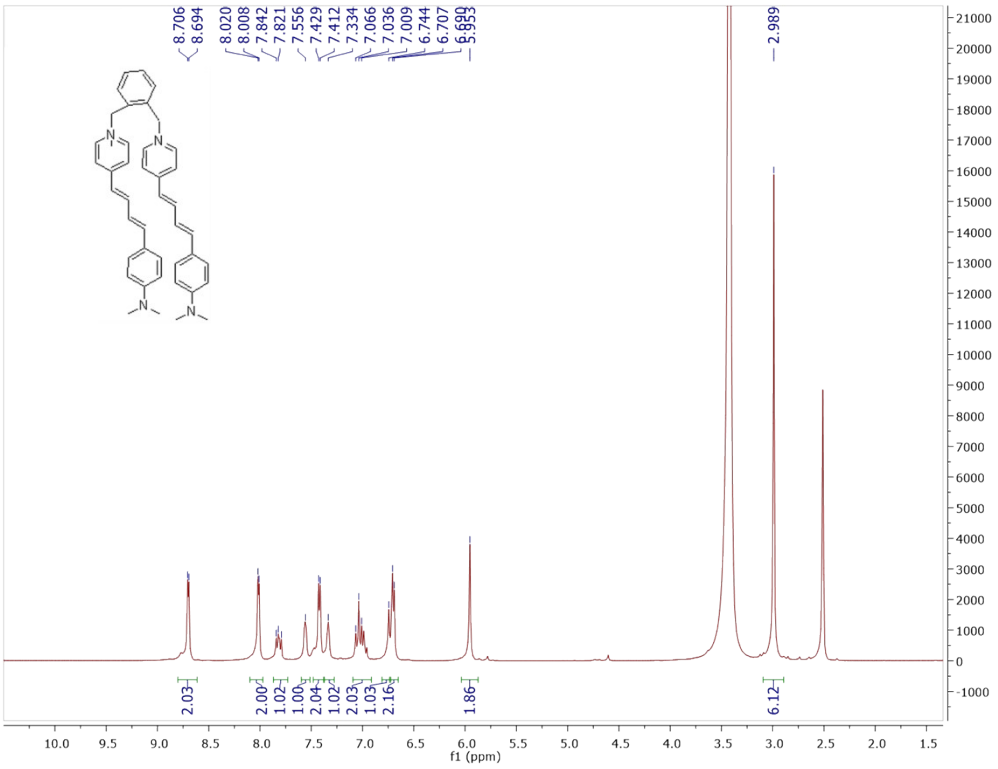


Figure S4. [1]HNMR spectrum of **NIR-SPM**

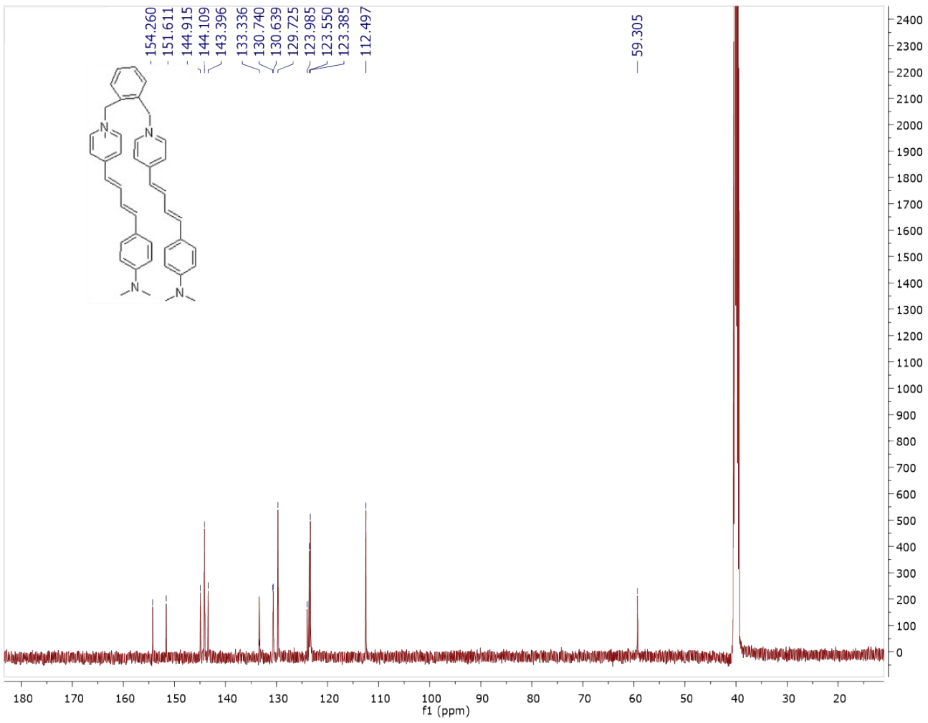


Figure S5: [13]CNMR spectrum of **NIR-SPM**

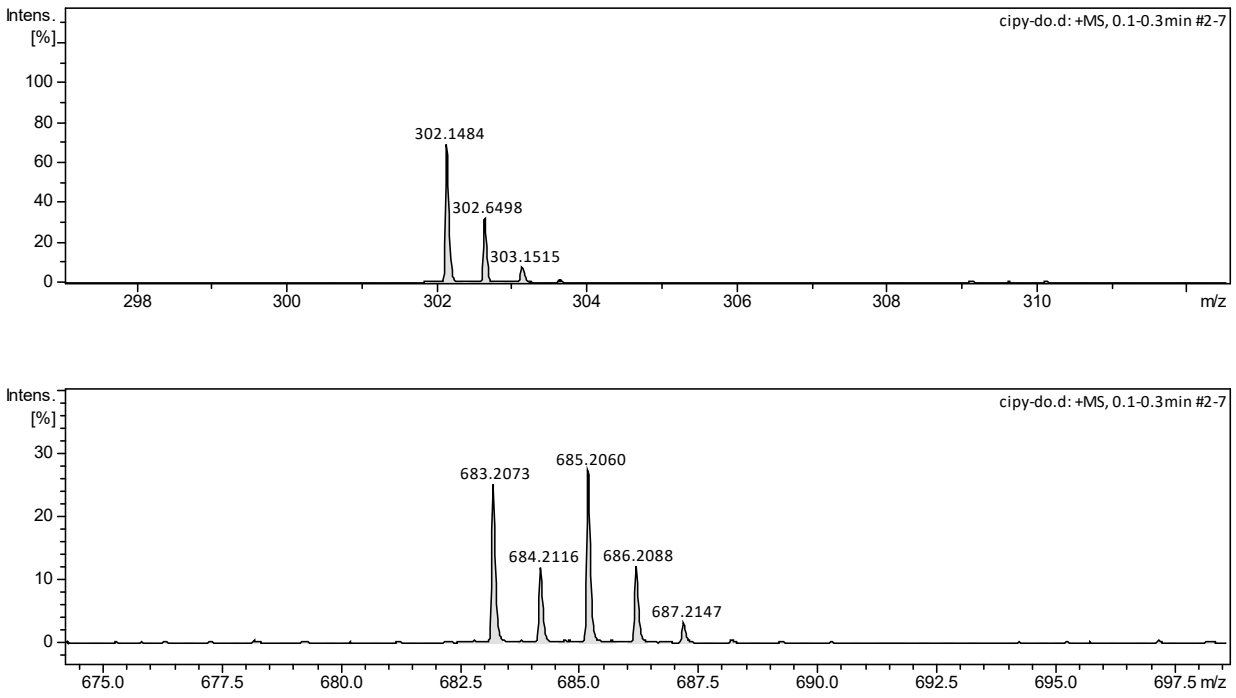


Figure S6: HRMS spectrum of **NIR-SPM**

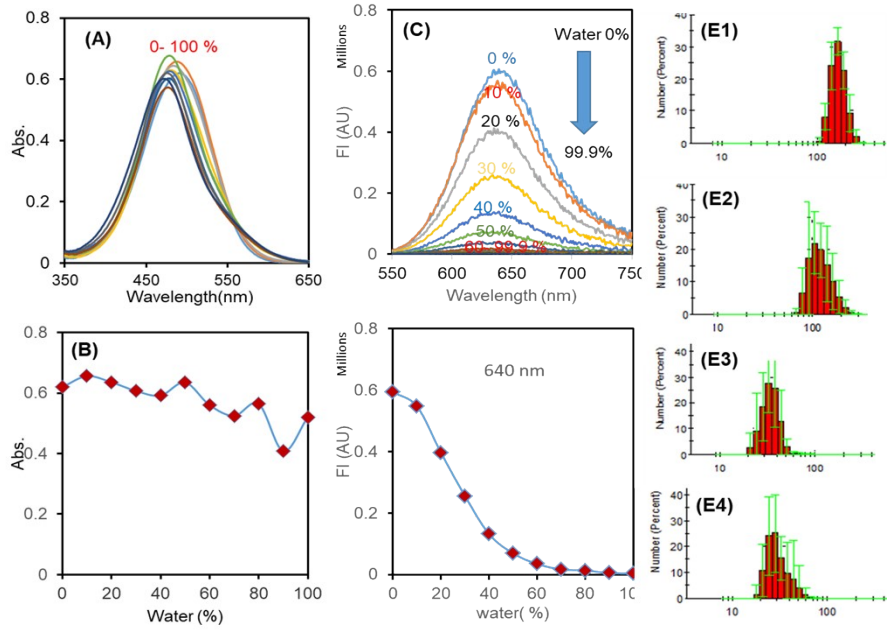


Figure S7. (A) Absorption spectra of **R-SPM** (10 μM) in DMSO-HEPES buffer binary mixtures; (B) The plot of f_w v/s absorption at 490 nm; (C) Fluorescence spectra of **R-SPM** (10 μM) in DMSO-HEPES buffer binary mixtures; (D) plot of f_w v/s emission at 640 nm; DLS spectra of **R-SPM** (10 μM) in (E1) 20% f_w; (E2) 40% f_w; (E3) 80% f_w; (E4) 99.9% f_w in DMSO

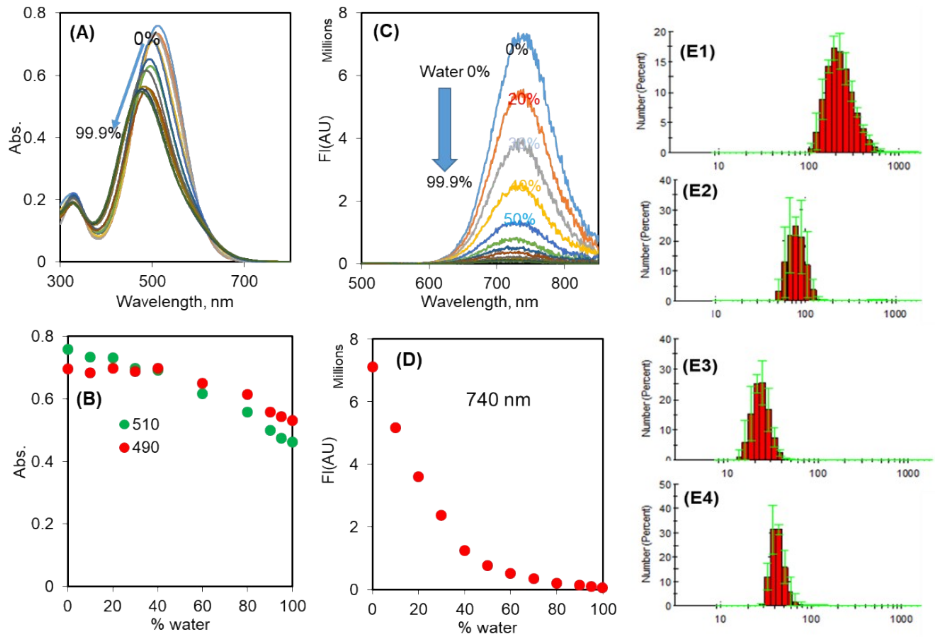


Figure S8. (A) Absorption spectra of **NIR-SPM** (10 μM) in DMSO-HEPES buffer binary mixtures; (B) The plot of f_w v/s absorption at 490 and 510 nm; (C) Fluorescence spectra of **NIR-SPM** (10 μM) in DMSO-HEPES buffer binary mixtures; (D) plot of f_w v/s emission intensity at 740 nm; DLS spectra of **NIR-SPM** (10 μM) in (E1) 40% f_w; (E2) 60% f_w; (E3) 80% f_w; (E4) 99.9% f_w in DMSO

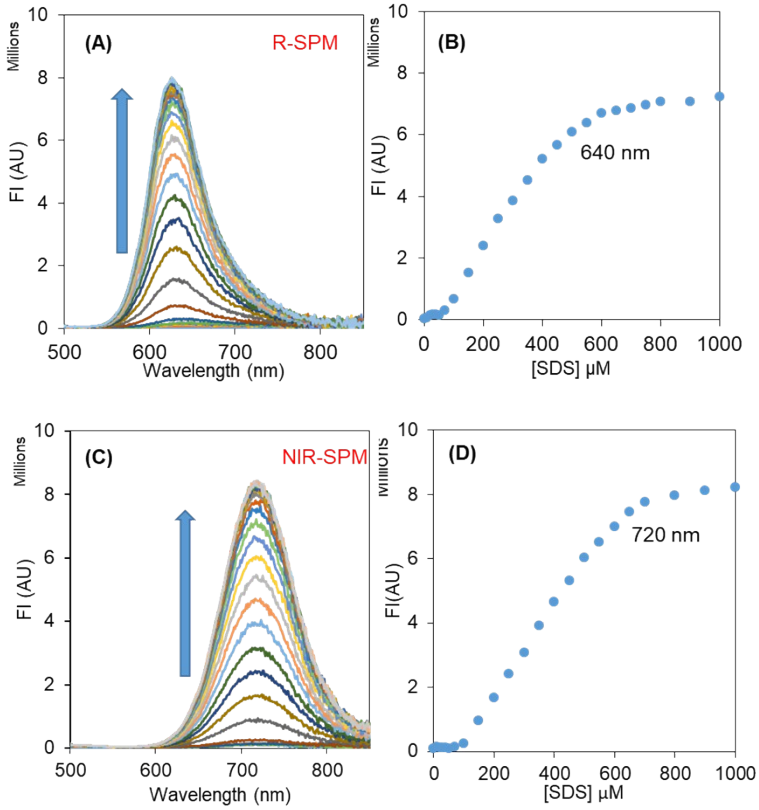


Figure S9. Effect of SDS concentration on interaction of spermine with R-SPM and NIR-SPM

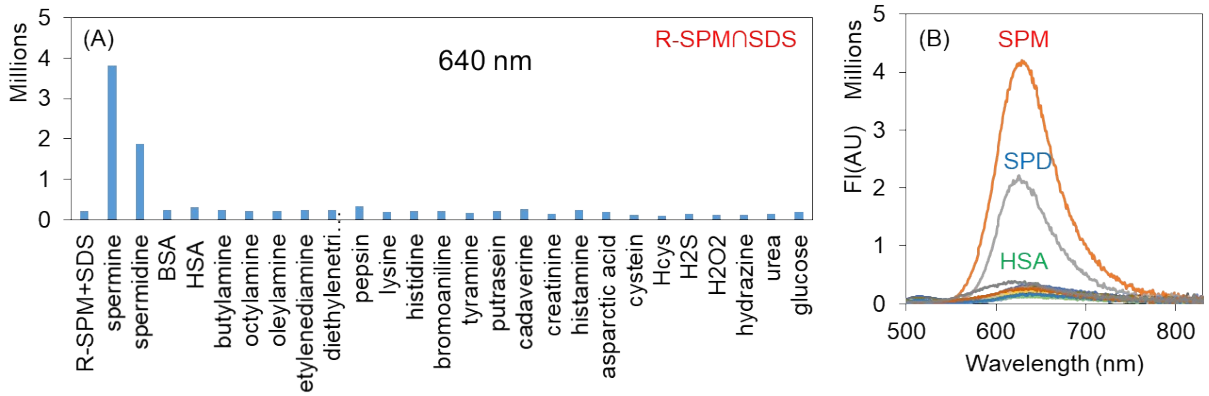


Figure S10. (A) The bar diagrams showing the change in fluorescence intensity on addition of 5 equivalents each of different species to **R-SPM∩SDS** (10 μM- 500 μM, HEPES buffer); (B) The fluorescence spectra of **R-SPM∩SDS** in the presence of different analytes.