Electronic Supplementary Material (ESI) for Analyst.

Supramolecular Self-Assembly of Nitro-incorporated Quinoxaline Framework: Insights into the Origin of Fluorescence Turn-on Response towards Benzene group of VOCs

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Experimental Section

General Information and Materials

All the reagents, solvents, starting materials, and different OVC analytes were procured from commercial purveyors and were used as received. All were of reagent grade. The solvents used were HPLC grade. For N.M.R. analyses, deuterated solvent [$(CD_3)_2SO$] was purchased from Sigma-Aldrich.

The UV-Visible absorption spectra were archived on a Perkin-Elmer Lamda-750 UV-Vis spectrophotometer using 10 mm path length quartz cuvettes in 250-700 nm wavelengths. Baseline correction was applied for all spectra.

Fluorescence emission spectra were documented on a Horiba Fluoromax-4 spectrofluorometer using a 1 cm path length of quartz cuvettes having a slit width of 3 nm at 298 K.

High-resolution mass spectrometry of **Probe 1** and **Probe 1**-Mesitylene was carried out on a Waters Q-ToF Premier mass spectrometer.

The solution-phase ^{1}H and ^{13}C Nuclear Magnetic Resonance spectra were recorded at 400 MHz using a Bruker Advances 400NMR instrument. The chemical shifts were reported in parts per million (ppm) with the deuterated solvents. The following abbreviations are used to delineate spin multiplicities in ^{1}H NMR spectra: s = singlet; d = doublet; t = triplet; q= quartet, m = multiplet.

Synthesis of Probe 1

To a suspension of 4-nitro ortho-phenylene diamine (153.14 mg, 1 mmol) in 25 ml glacial acetic acid, isatin (176.41 mg, 1 mmol) was added portion-wise at room temperature. The reaction mixture was allowed to reflux overnight. After stirring for 24 hours, it was cooled to room temperature, and crushed ice was added slowly to get the precipitate. The precipitate was then filtered off and washed several times with methanol/acetic acid followed by ether to obtain the pure product.

Probe 1: brown solid (85% yield); HRMS (*m/z*): calculated for $C_{14}H_8N_4O_2$ $[M+H]^+$: 265.0726; found: 265.0723; ^{1}H NMR (600 MHz, DMSO-d_6): 9.045 (s, 1H), 8.578 (s, 1H), 8.444 (d,1H) , 8.306(d, 1H), 8.257 (d, 1H), 7.9561 (d, 1H), 7.698-7.660 (m, 1H), 7.382-7.349 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6):148.61, 146.39, 145.00, 143.22, 138.16, 135.48, 133.76, 130.41, 125.21, 124.10, 116.63, 114.92, 111.91, 111.11; FT-IR (KBr pellets, cm^{-1}):

3091(N-H stretching), 2924(C-H stretching), 1719(C-H bending), 1618(C=N), 1521(N-O assymetric stretch), 1337(N-O symmetric stretch), 750(N-H wagging).

General Procedure for UV-VIS and Fluorescence Spectroscopic Studies

Stock solutions of different VOCs (50×10^{-3}mol L^{-1}) were prepared in DMSO. A stock solution of **Probe 1** (1×10^{-3}mol L^{-1}) was prepared in DMSO. For fluorescence selectivity experiments, the solution of **Probe 1** was then diluted to 2×10^{-6} mol L^{-1} with Millipore water by taking only 4μL of stock solution and making the final volume 2 mL. In fluorescence titration experiments, a quartz optical cell of 10mm pathlength was filled with a 2.0 mL solution of **Probe 1** to which various VOC's stock solutions were gradually added using a micropipette. For fluorescence measurements, **Probe 1** was excited at 400 nm, and emission was procured from 420 nm to 650 nm.

Estimation of the Apparent Binding Constant

Probe 1 with a sufficient concentration of 2μM in water was titrated with varying mesitylene concentrations. Thus the apparent binding constant for forming the **Probe 1**-mesitylene complex was assessed utilizing the Benesi−Hildebrand (B−H) plot (**Equation 1**).

$$1/(I-I_0) = 1/\{K(I_{max}-I_0)\ C\} + 1/(I_{max}-I_0) \qquad (1)$$

I_0 is the emission intensity of **Probe 1** at maximum (λ = 481 nm), and I is the recorded emission intensity at that particular wavelength in the presence of a specific concentration of the analyte (C). I_{max} is the maximum emission intensity value obtained at λ = 481 nm during titration with varying analyte concentrations. K is the apparent binding constant (M^{-1}) and was determined from the linear plot's slope.

Detection Limit

The detection limit was evaluated based on the fluorescence titration changes for mesitylene. **Probe 1**'s fluorescence emission spectrum was computed ten times, and the standard deviation of the blank measurement was obtained. The fluorescence emission at 481 nm was plotted as a concentration of mesitylene to gain the slope. The detection limits were calculated using the following equation:

$$\text{Detection limit} = 3\sigma/k \qquad (2)$$

where σ is the standard deviation of blank measurement, and k is the slope between the fluorescence emission intensity versus [mesitylene]. The conversion to ppm unit was done considering Mol. Wt. of mesitylene 120.19 $gmol^{-1}$.

Field Emission Scanning Electron Microscope (FESEM) Studies

Morphology of **Probe 1**, **Probe 1-**mesitylene complex were imaged separately using Gemini 300 FESEM (Carl Zeiss) instrument. The samples were prepared by drop-casting (2μM), the DMSO/Water mixture on Al-foil wrapped coverslip, then coated with Au and dried under vacuum before the imaging.

Dynamic light scattering measurement

Dynamic light scattering (D.L.S.) experiments were performed on Malvern Zetasizer Nano Z.S. instrument equipped with a 4.0 mW He–Ne laser running at a wavelength of 633 nm. The samples and the background were measured at room temperature (25 °C) at a scattering angle of 173°. D.L.S. experiments were executed with optically clear solutions of **Probe 1** (2 μM) in water with 50 equivalents of mesitylene. The solution was equilibrated for 60 minutes before taking the measurements.

Fluorescence Microscopy

The freshly prepared samples of **Probe 1** (2 μM) and Probe **1-**mesitylene complex (**Probe 1** mixed with 50 equivalents of mesitylene)glass slide and were entirely dried at room temperature, followed by image acquisition using a Fluorescence microscope (Eclipse Ti-U, Nikon, U.S.A.) with a blue filter.

Measurement of fluorescence lifetime

Fluorescence lifetimes were evaluated utilizing the time-correlated single-photon counting (TCSPC) method in the Edinburgh Instrument Life-Spec II spectrometer. The samples (**Probe 1** and **Probe 1-**mesitylene) were excited at 400 nm keeping the emission wavelength at 481 nm using a pulsed diode laser. The fluorescence decays were surveyed by the re-convolution method using the FAST software provided by Edinburgh Instruments.

Photoluminescence Quantum Yield

We had pursued the Petite Integrating Sphere method to determine the quantum yield by Horiba Jobin Yvon Fluoromax-4 Spectrofluorometer. It was determined in 100% aqueous medium for **Probe 1** (2 μM) and **Probe 1-**mesitylene complex (**Probe 1** mixed with 50 equivalents of mesitylene), keeping λ_{ex} at 400 nm. To determine the quantum yield, the equation we employed was (as per the instruction written on the official website of Horiba),

$$\Phi = [(E_c - E_a) / (L_a - L_c)] \qquad (3)$$

Where E_c = Emission of the sample, E_a = Emission of the blank, L_c = Scatter of the sample & L_a = Scatter of the blank.

Theoretical investigations (DFT study)

DFT optimizations of **Probe 1** and **Probe 1-**mesitylene complex were accomplished with the RB3LYP/ 6-31G (d,p) method basis set using the Gaussian 09 program.

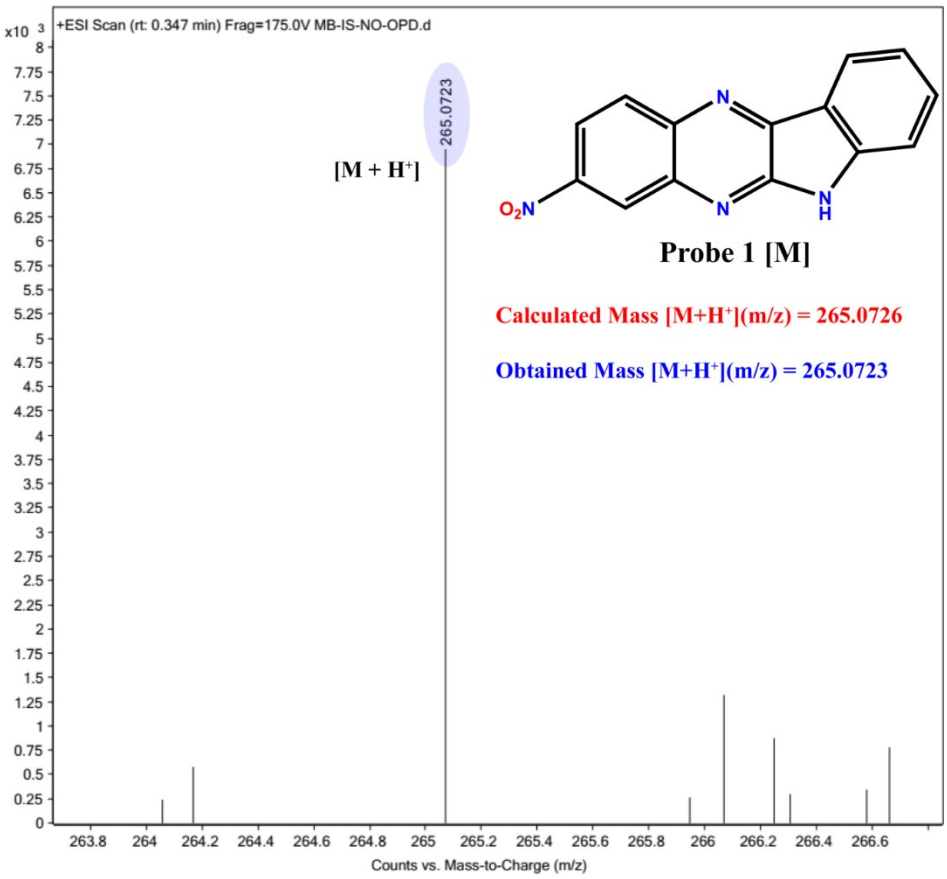


Figure S1: HRMS spectra of **Probe 1** in 1:1 water-acetonitrile in positive ionization mode.

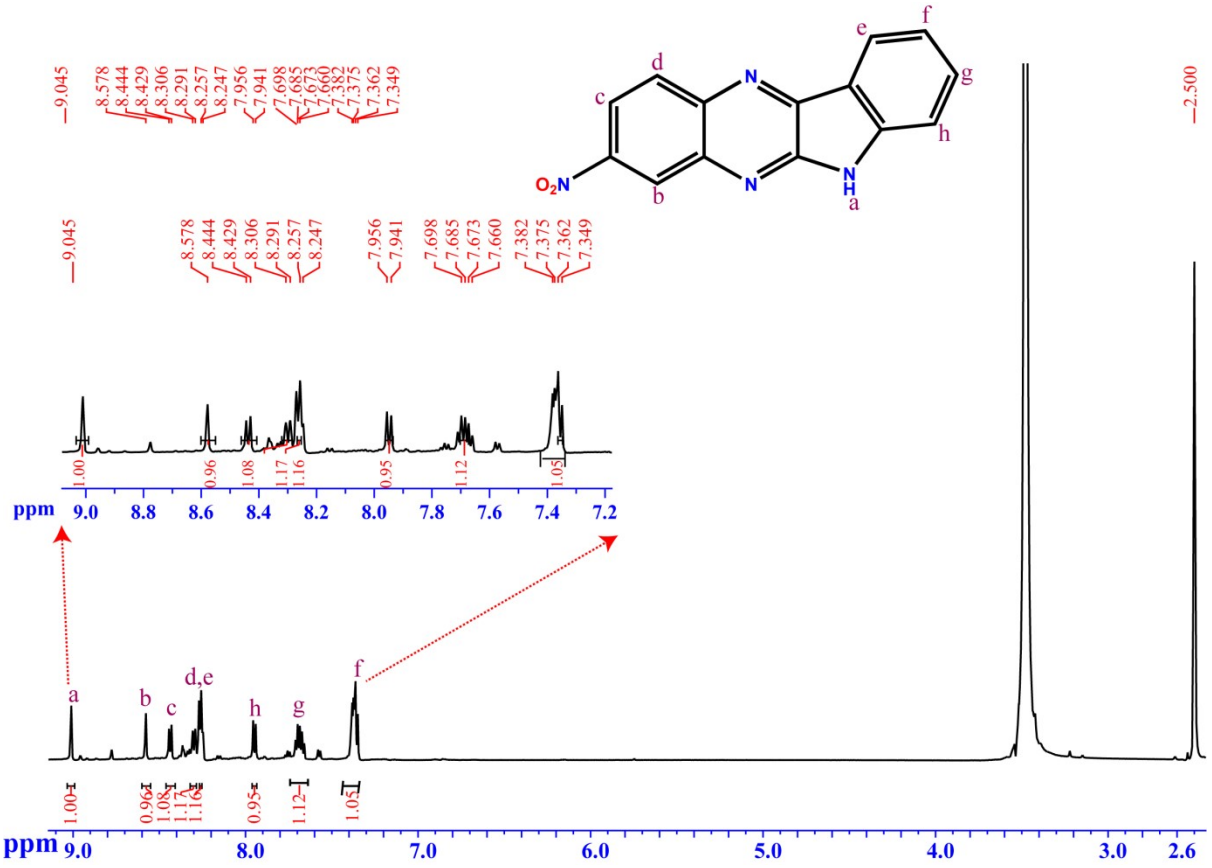


Figure S2: ^{1}H N.M.R. of **Probe 1** in DMSO-d_6 at room temperature.

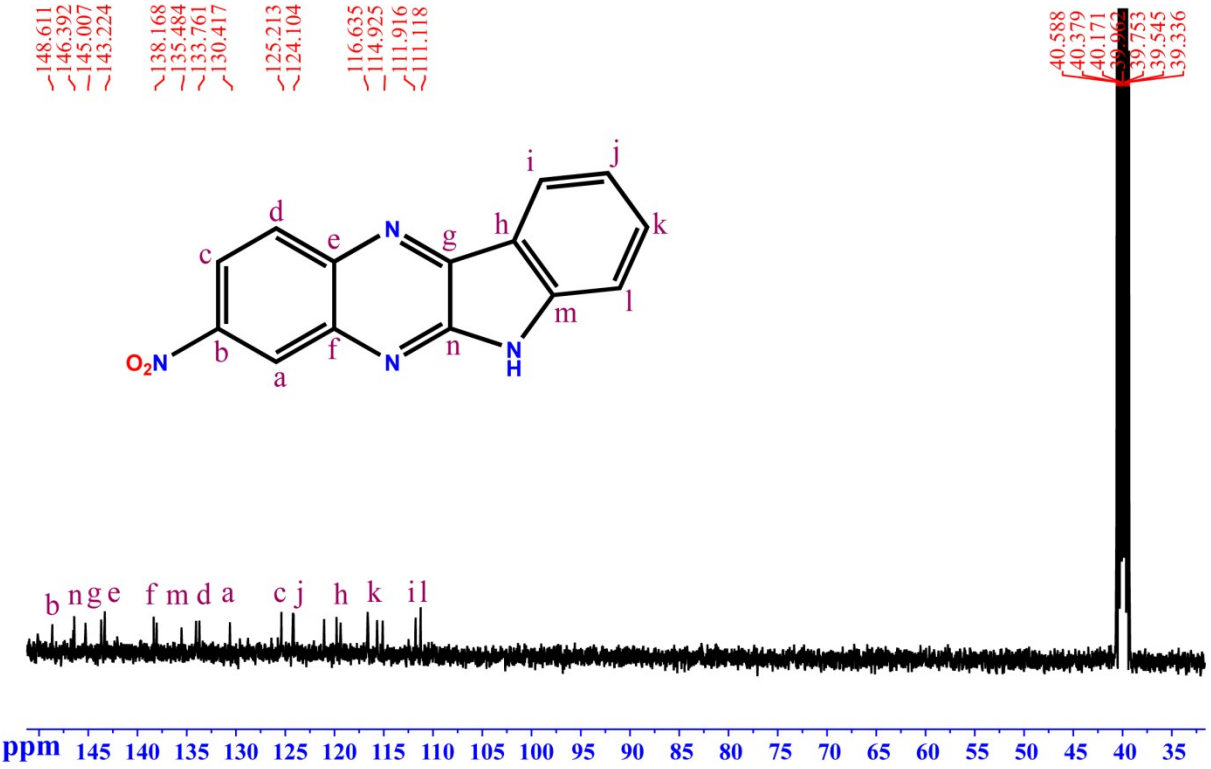


Figure S3: ^{13}C N.M.R. of **Probe 1** in DMSO-d_6 at room temperature.

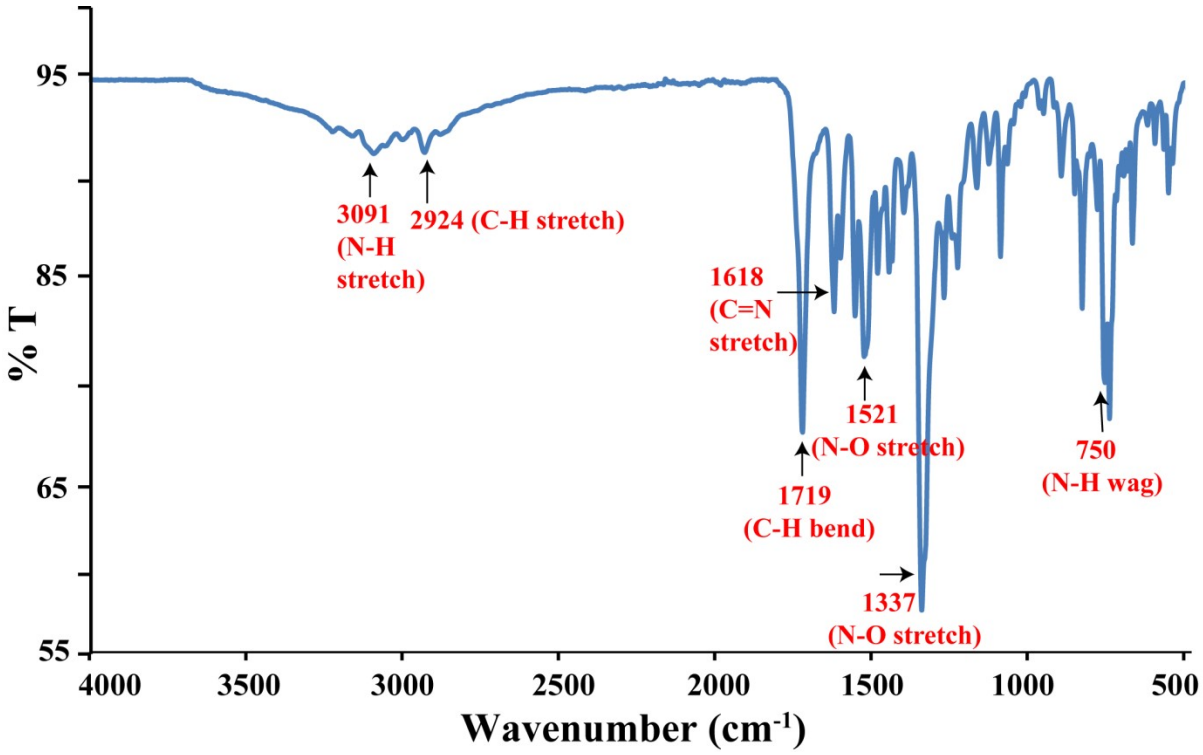


Figure S4: FTIR spectrum of **Probe 1** recorded in KBr pellet at room temperature.

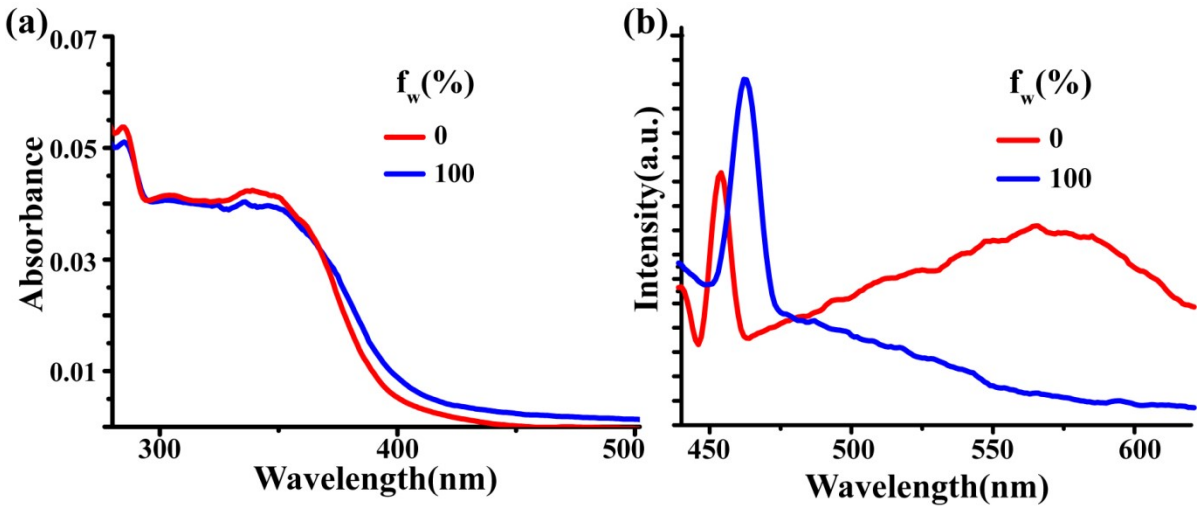


Figure S5: (a) UV-Vis and (b) Fluorescence changes of **Probe 1** (2 μM) in different water fractions at room temperature.

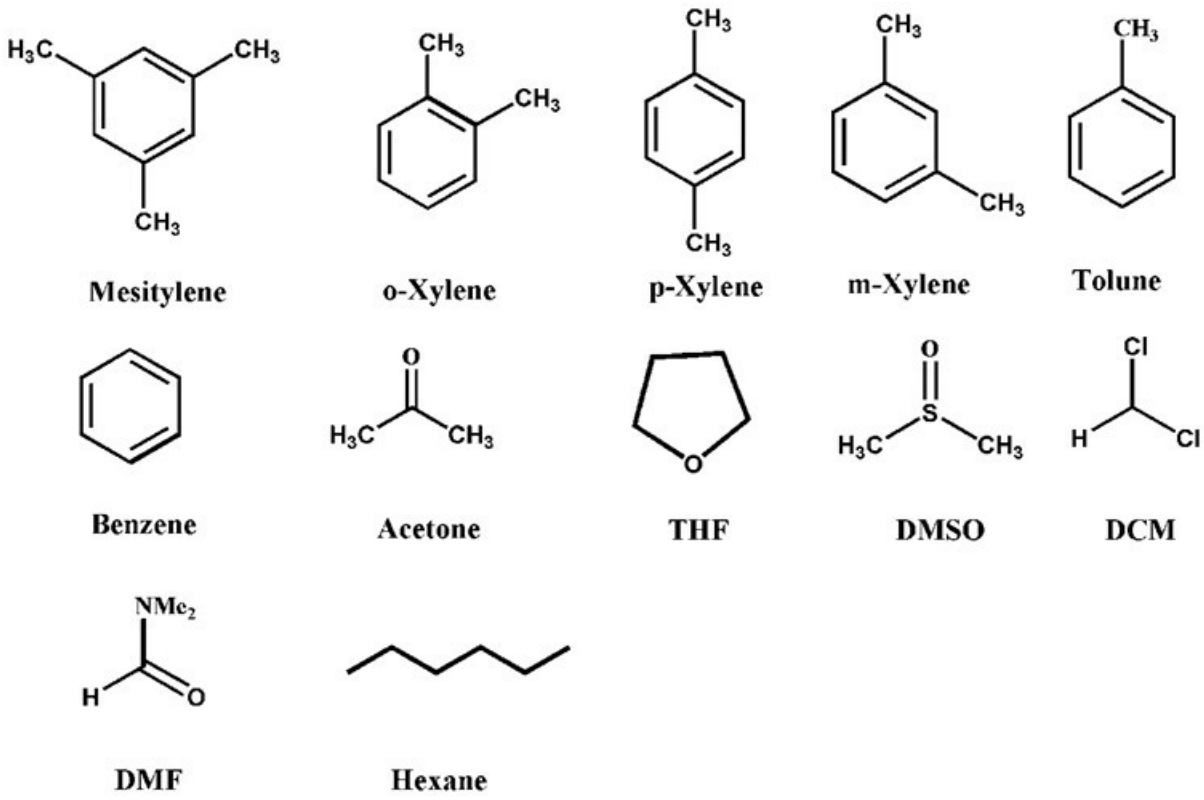


Figure S6: Chemical structures of different aromatic and non-aromatic V.O.C. compounds used in our study.

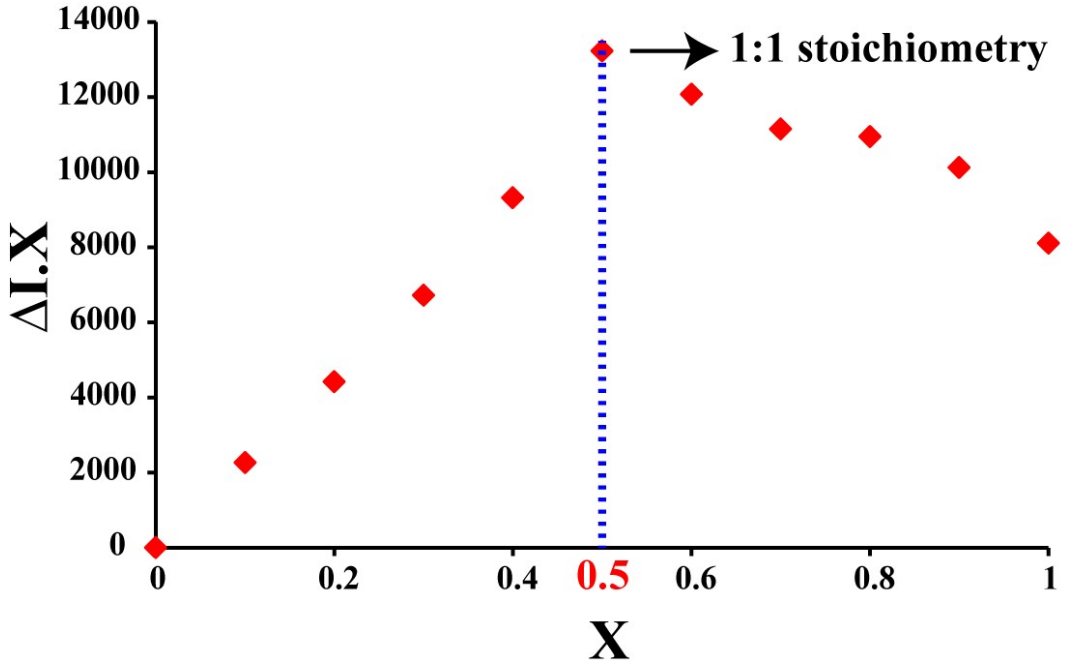


Figure S7: Job's plot calculation to determine the binding stoichiometry between **Probe 1** and Mesitylene in 100% solution (λ_{ex} = 400 nm).

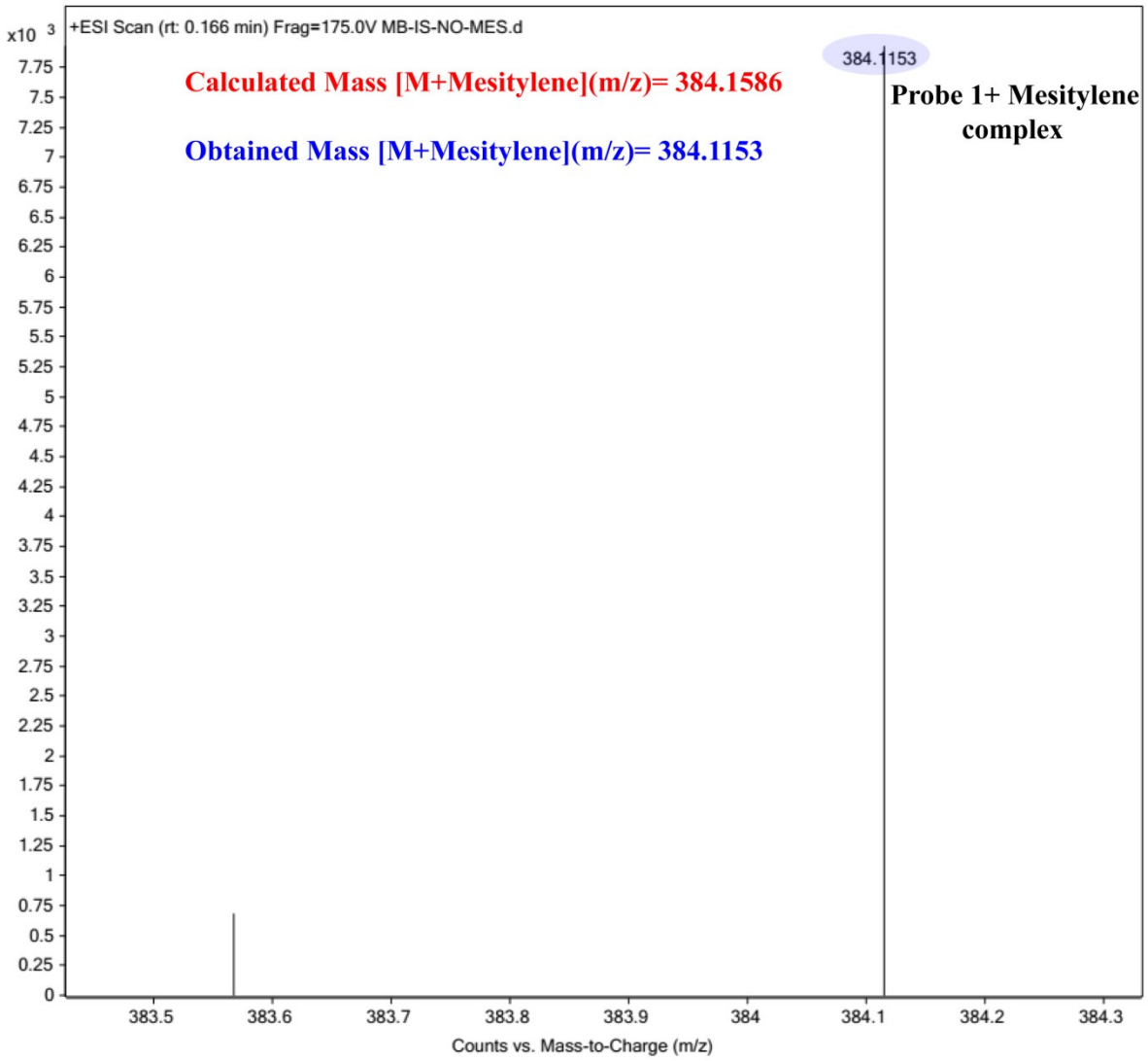


Figure S8: HRMS spectra of **Probe 1** in 1:1 water-acetonitrile in the presence of Mesitylene in positive ionization mode.

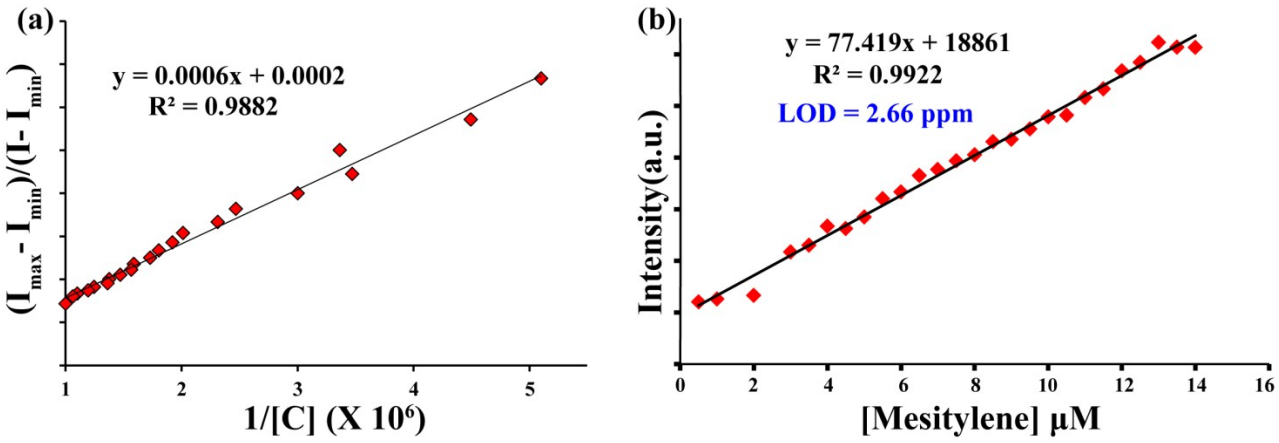


Figure S9: (a) Determination of binding constant of **Probe 1** for Mesitylene in aqueous medium using the Benesi-Hildebrand method considering 1:1 interaction. (b) Fluorescence emission intensity of **Probe 1** at 481 nm vs. Mesityleneconcentration to calculate the detection limit.

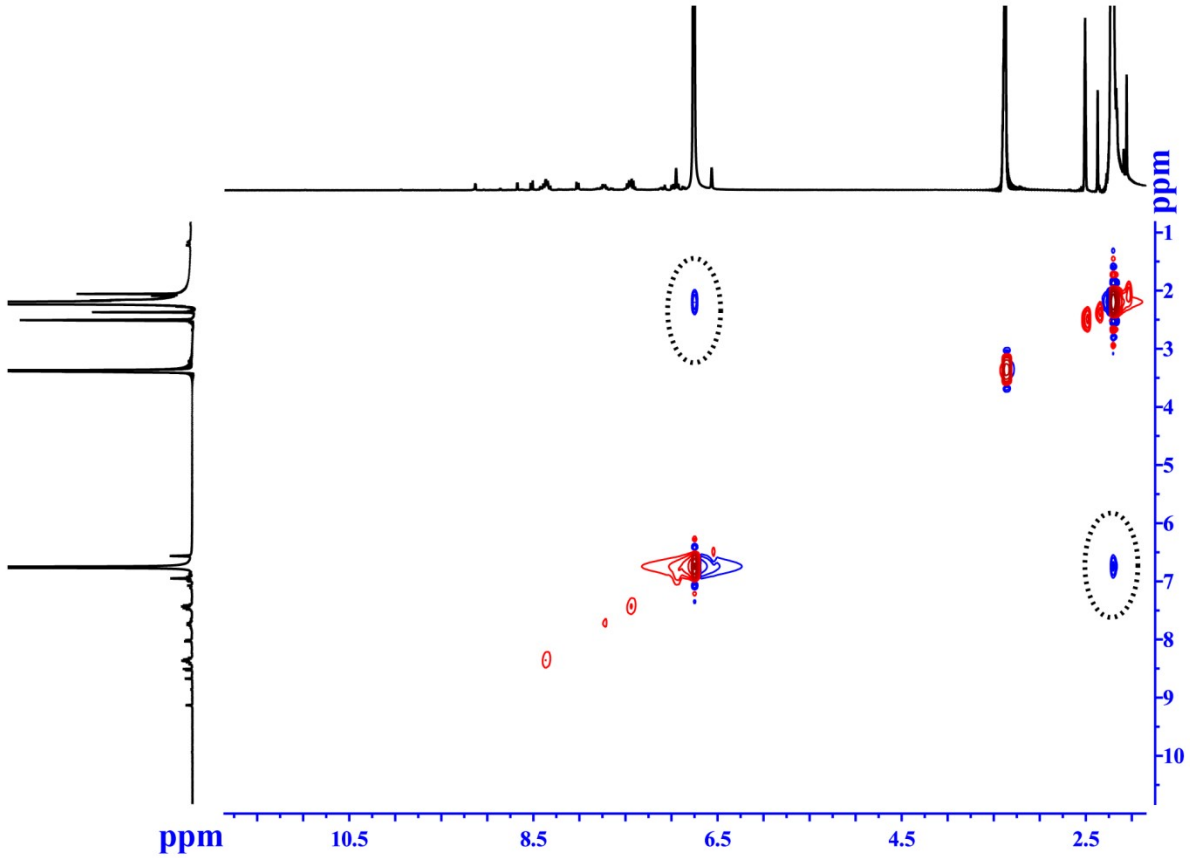


Figure S10: NOESY NMR spectra of **Probe 1**-Mesitylene.

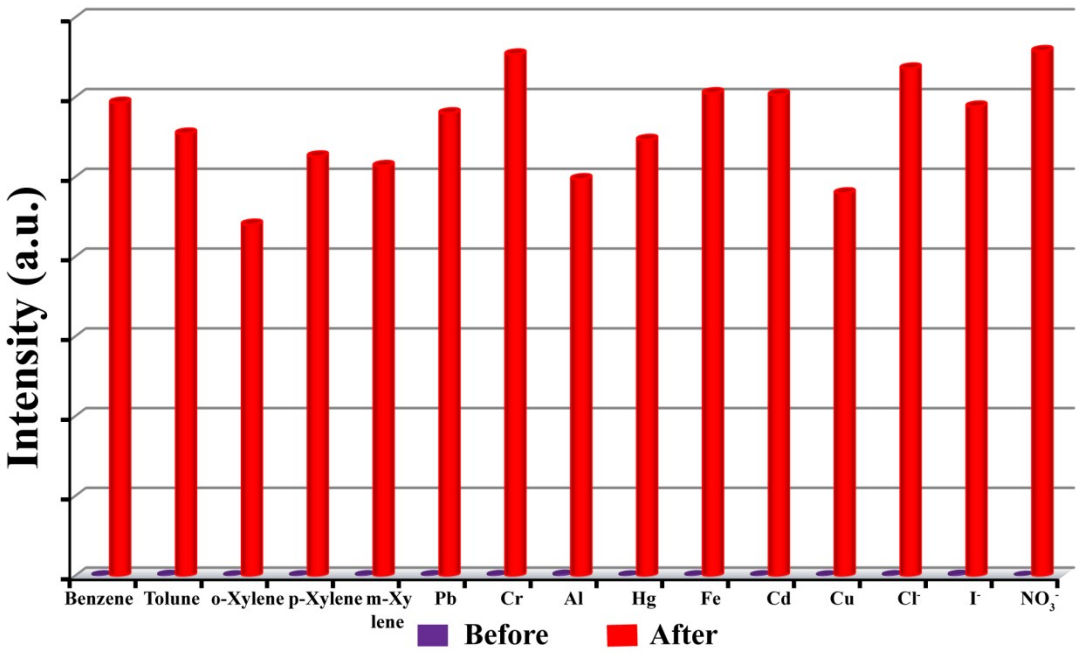


Figure S11: Fluorescence response of **Probe 1** (2μM) to various analytes, before (violet bars) and after (red bars) addition of Mesitylene.

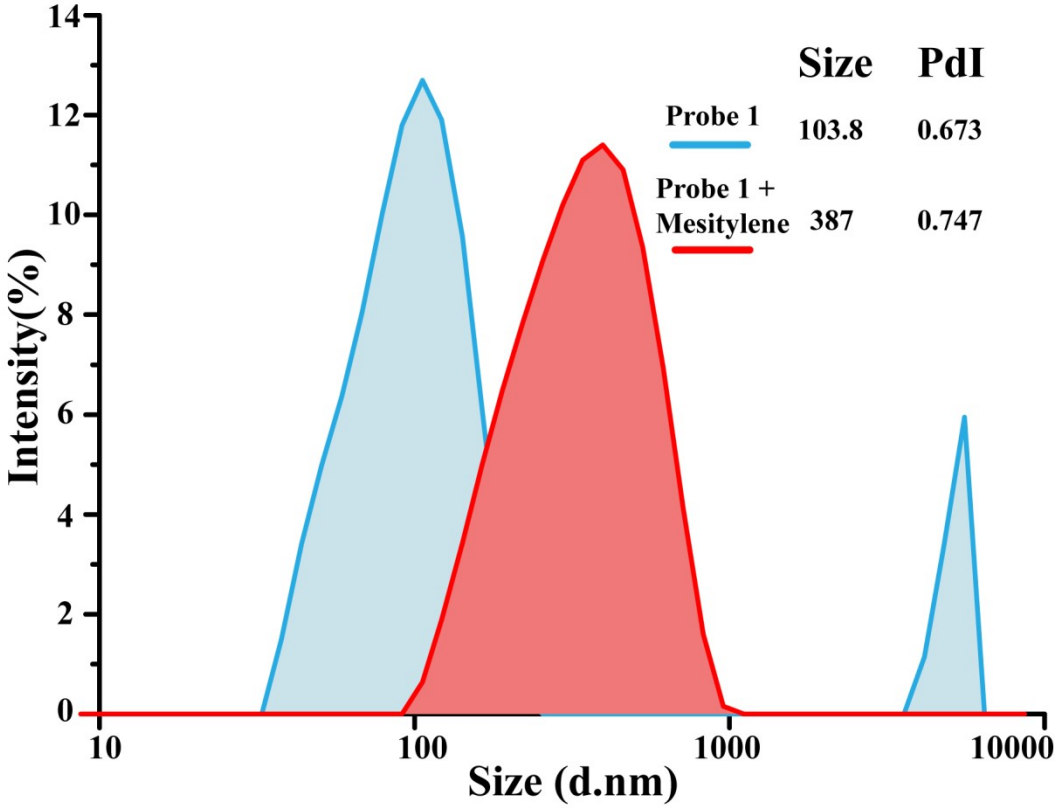


Figure S12. D.L.S. spectra of **Probe 1**(2 μM) and **Probe 1** + Mesitylene in the aqueous medium.

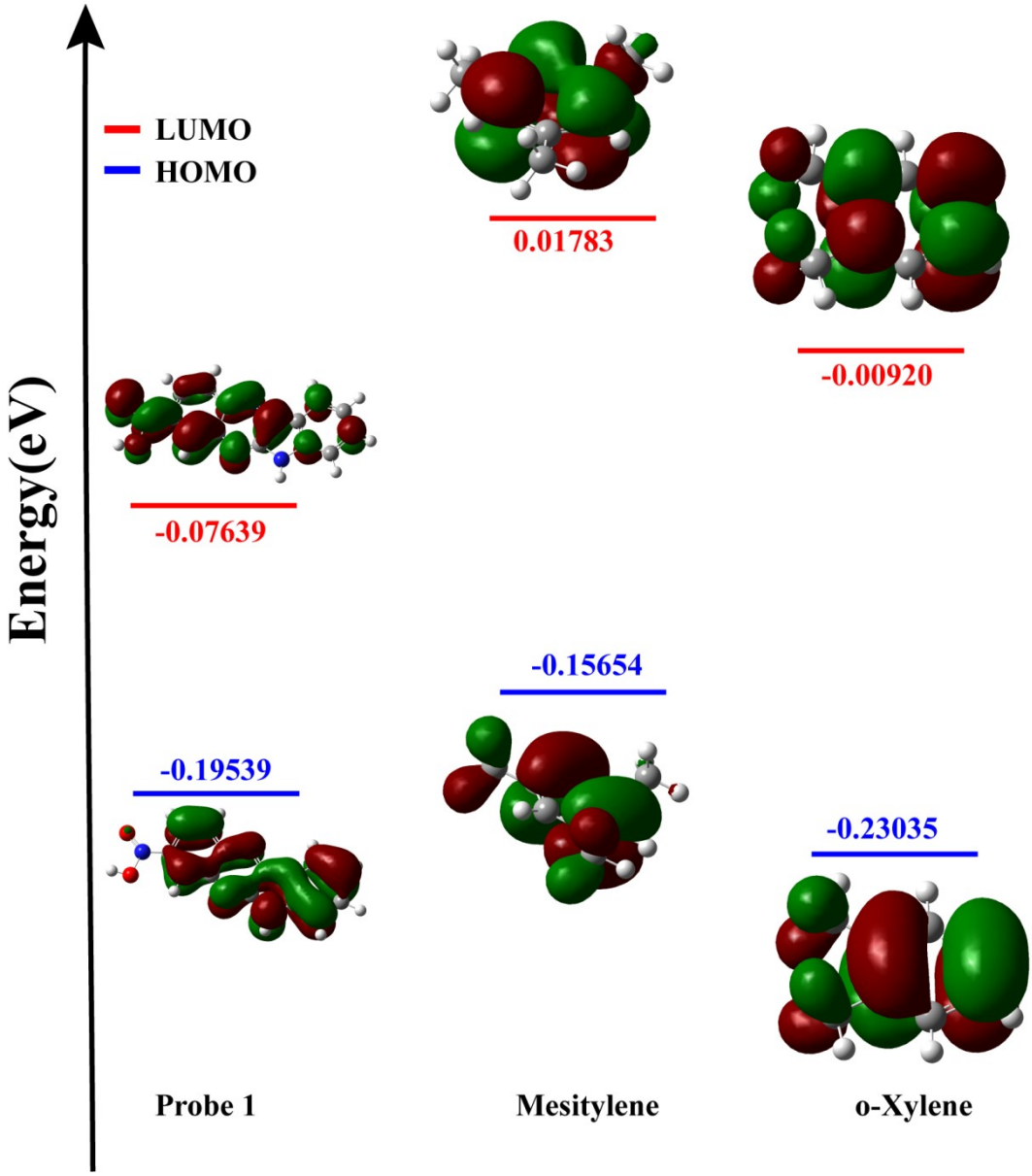


Figure S13: DFT calculated HOMO-LUMO energy profiles of **Probe 1**, Mesitylene and o-Xylene.

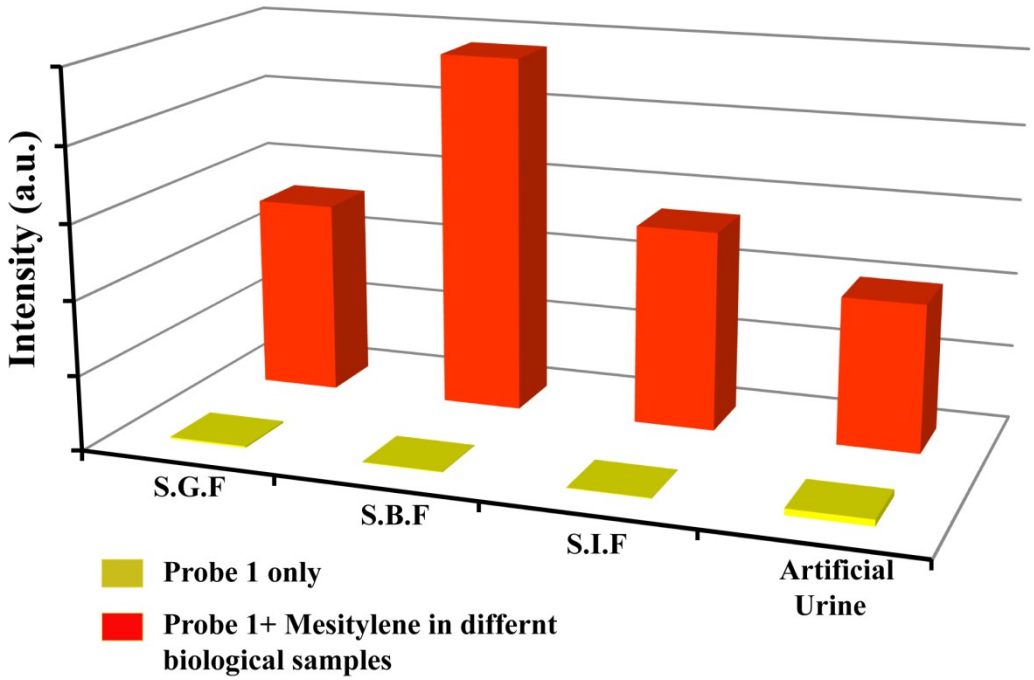
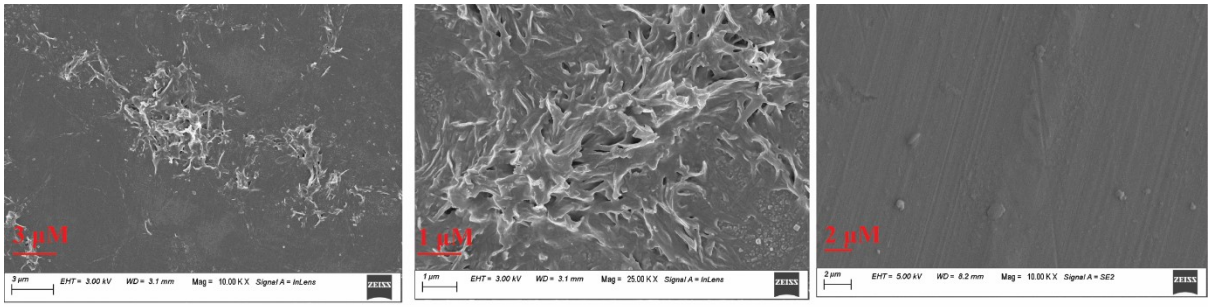


Figure S14: Changes in the emission intensity of **Probe 1** (2 μM) at 481 nm in the presence of excess Mesitylene in bio-fluidic samples [S.G.F- Simulated Gastric Fluid (pH~2.0), S.B.F- Simulated Body Fluid (pH~7.4), S.I.F- Simulated Intestinal Fluid (pH~8.0) and Artificial Urine].



Probe 1+ o-Xylene Probe 1+ tolune Probe 1+ benzene

Figure S15: FESEM images of **Probe 1** with different VOCs.

Table S1: Fluorescence lifetime values of **Probe 1** (2μM) and **Probe 1**-Mesitylene in aqueous medium.

Sample	B_1	B_2	B_3	a_1	a_2	a_3	τ_1	τ_2	τ_3	$\langle\tau\rangle$ (ns)	χ^2
Probe 1	0.0198	2.0809	-	0.7220	0.2779	-	0.818	0.003	-	0.591	1.089
Probe 1+ Mesitylene	0.0836	0.0153	0.0018	0.6667	0.2465	0.0866	1.136	2.290	6.955	1.924	1.024

Table S2: Comparative analysis for detection of Mesitylene in natural water samples.

	Fluorescence Spectroscopy			UV-Visible Spectroscopy		
Sample	**Added (µM)**	**Detected (µM)**	**Recovery (%)**	**Added (µM)**	**Detected (µM)**	**Recovery (%)**
Industrial wastewater	5.0	5.30	103	5.0	6	105
Lake water	5.0	4.00	85	5.0	4.8	88
River water	5.0	4.8	93	5.0	5.1	101

Table S3: Comparison of the detection limits achieved till now along with the receptor and solvent system used for the detection of VOCs

Sl No.	References	Receptor	Analytical Methods	Medium Used	L.O.D. (ppm)
1.	**Present work**	**Quinoxaline derivative**	**Fluorescence Spectroscopy**	**100% Aqueous medium, Vapor, Paper Strip**	**2.66**
2.	Sensors & Actuators: B. Chemical, 2020, 311, 127904	Poly(maleic anhydride-alt-1-octadecene) (PMAO) nanoparticles	Fluorescence Spectroscopy	Aqueous medium	7
3.	Chem. Sci., 2018, 9, 1892–1901	Organoboron polymer	Fluorescence Spectroscopy	Vapor	3.7
4.	Molecules 2017, 22, 1306	Polystyrene films	Fluorescence Spectroscopy	Vapor	150
5.	Anal. Chem., 2017, 89, 3814	Perylene monoimide	Fluorescence Spectroscopy	Vapor	8
6.	Chem. Mater. 2016, 28, 7889	TPE based M.O.F.	Fluorescence Spectroscopy	Solution	-
7.	Scientific Reports, 2015, 5, 12462	Molecularly imprinted polymer	Potentiometry	Vapor	3.5
8.	J. Am. Chem. Soc. 2014, 136, 7241	2D M.O.F.	Fluorescence Spectroscopy	Solution	-
9.	Chem. Mater. 2015, 27, 1465	Coordination Polymer	Raman Spectroscopy	Solution/ Vapor	500
10.	Chem. Commun., 2011, 47, 1160–1162	Silver(I) pyrazolate	Fluorescence Spectroscopy	Vapor	-