Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information

Converging Optical and Electrochemical Detection Strategies for Multimodal Hydrazine Sensing: Insights into Substituent-Driven Diverse Response

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EXPERIMENTAL SECTION

1.1 General. All chemicals (solvents, reagents, and chemicals) were purchased from the best-known local chemical suppliers and used without further purification. Solvents were distilled and dried before use. FTIR spectra were recorded on a Perkin-Elmer FT-IR Spectrum BX system and were reported in wave numbers (cm^{-1}). On the other hand, ^{1}H NMR and ^{13}C NMR spectra were recorded with a Bruker Advance DRX 400 spectrometer operating at 400 and 100 MHz for ^{1}H and ^{13}C NMR spectroscopy, respectively. Chemical shifts were reported in ppm downfield from the internal standard, tetramethylsilane (TMS). Mass spectra were recorded on a Micromass Q-TOF Micro TM spectrometer.

1.2 Spectroscopic studies. The UV-vis spectroscopic studies were recorded on a JASCO (model V-650) UV-Vis spectrophotometer. The slit width for the experiment was kept at 5 nm. Sensing was carried out by adding requisite amounts of hydrazine to water (1 % DMSO) solutions of probe **1** (1×10^{-6} M). On the other hand, fluorescence experiments were performed in FluoroLog-TM (Horiba Scientific). The slit width for the fluorescence experiment was kept at 5 nm (excitation) and 5 nm (emission) and the excitation wavelength was set at 435 nm.

1.3 Fluorescence Titrations: Stock solutions of 1 mM of **1** were prepared in DMSO. A standard fluorescence cuvette (10 mm) was filled with 2 mL of the respective solvent, to which 20 µL of probe **1** (1 mM) was added. An initial fluorescence spectrum was recorded using $\lambda_{ex} = 435$ nm. Subsequently, increasing concentrations of hydrazine were added to the probe solution, and the fluorescence was recorded. All the spectroscopic studies have been repeated for three times to determine the errors in the measurements.

1.4 Detection limit determination. The method used for the calculation of the detection limit is known as the blank variability method. In this method, the calibration curve was prepared by recording fluorescence spectra of **1** in different amounts of hydrazine.

From the equation obtained from the calibration plot, the added hydrazine concentrations were calculated. Then another calibration curve was drawn between the C_{real} (added hydrazine, µM) vs. $C_{calc.}$ (Calculated amount of hydrazine, µM). This afforded a value of the slope (b).

The fluorescence spectra of **1** were taken as blank readings. A total 10 replicates of the blank were measured. The standard deviation from the blank readings was calculated by fitting the fluorescence reading into the equation obtained from the first calibration curve (titration spectra). Using this standard deviation value, we calculated the decision limit by the following equation.

$L_C = t_C \times s \times (1 + 1/N)1/2$..... ..... (1)

where, N = the number of blank replicates taken; the value of t_c for 10 blank readings is 1.833; and s = the standard deviation value.

The detection limit (L_D) was calculated as double the decision limit obtained,

$L_D = 2\ L_c$ …… (2)

In concentration terms, the detection limit appeared as,

$x_D = 2 \times C = 2\ L_C / b$ …… (3)

where, b = slope of the second calibration curve (Creal vs. Ccalc.).

1.5 ^{1}H NMR Studies: ^{1}H NMR titration studies of compound **1** (5 mM) were performed with hydrazine (1.0 equiv.) in DMSO-D_6. The spectra were recorded using identical parameters.

1.6 Stoichiometry determination by Job plot: Job plot is a method of continuous variation for determining the stoichiometry of interaction between the two species. The total molar concentration of the two binding species (here, **1** with hydrazine) was kept constant (1 x 10^{-6} M) and the mole fraction was varied. Further, the changes in fluorescence were plotted against the mole fraction. The maxima or minima thus obtained gave the stoichiometry of interaction. In all cases, we have plotted ΔI*[hydrazine] vs [probe] / {[hydrazine] + [probe]}. Where, $\Delta I = I - I_0$, I = fluorescence of **1** after the addition of hydrazine at specific wavelengths, and I_0 = fluorescence of **1** without hydrazine. [probe] / {[hydrazine] + [probe]} is the mole fraction of the probe molecule in the mixture (Probe = **1**).

1.7 Electrochemical studies

The electrochemical experiments were performed by an Auto lab potentiostat PGSTAT128 N using a conventional three-electrode system. The electrodes were Glassy carbon electrode (GCE) as a working electrode, a Pt-wire as an auxiliary electrode, a saturated Ag/AgCl as a reference electrode. The polished GCE was of 3 mm in diameter with 0.07 cm^2 surface area. The electrochemical reaction was recorded by cyclic voltammetry (CV) technique at 0.05 V/s scan rates in Acetonitrile containing 0.1 M Tetra butyl ammonium perchlorate (TBAP). The energies of HOMO and LUMO levels were determined from the onset potentials for reduction (E_{red} onset) and oxidation (E_{ox} onset), respectively, and the CV was recorded in the potential range 2 mV to -1 mV Vs Ag/AgCl.

Electrochemical impedance studies (EIS) was carried out using Biologic Sp-150 with the conventional three electrode system over a frequency range of 10 kHz to 10 mHz. The three electrode system include Glassy carbon electrode (GCE) as a working electrode, a Pt-wire as an auxiliary electrode, a saturated Ag/AgCl as a reference electrode. Impedance of all samples were in Acetonitrile containing 0.1 M Tetra butyl ammonium perchlorate (TBAP).

The HOMO and LUMO values are derived from the equation as

$E_{HOMO} = -e\,[E_{ox\ onset} + 4.741]$ eV …….…… (1)

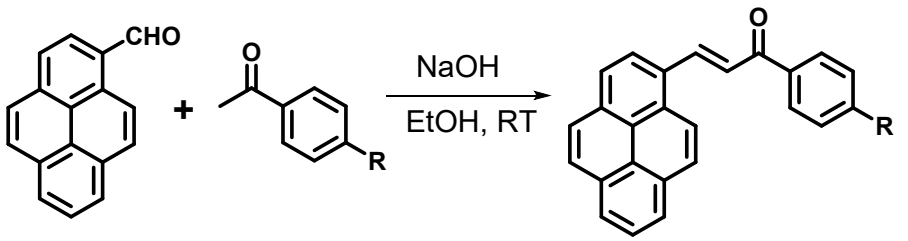
$E_{LUMO} = -e\,[E_{red\ onset} + 4.741]$ eV. ……..(2)

The fractional surface coverage (ɵ) is calculated by the equation (3)

$ɵ = 1 - R_{ct(hydrazine)}/R_{ct(probe)}$ ……(3)

where $Rct_{(hydrazine)}$ is the electron transfer resistance value of different concentrations of hydrazine, $Rct_{(probe)}$ is the electron transfer resistance value of probe.

SYNTHESIS



Compound 1 : R = NO_2
Compound 2; R = Cl

Synthetic scheme of compound **1** and **2**

Synthesis of Compound **1** : The synthesis was performed by referring to the procedure reported in literature.[1] Briefly, 4-Chloroacetophenone , 1-pyrenecarboxaldehyde and 3 M NaOH in ethanol was stirred at room temperature for 2-3 hours. The resulting solid was collected by filtration and recrystallized from ethyl acetate/acetic acid (v/v ¼ 1 : 1) to get orange crystals. Yield: 70%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.06 (d, *J* = 15.4 Hz, 1H), 8.55 (d, *J* = 9.3 Hz, 1H), 8.45 (d, *J* = 8.1 Hz, 1H), 8.41 (d, *J* = 12.4 Hz, 1H), 8.28 – 8.24 (m, 4H), 8.12 – 8.08 (m, 4H), 8.06 (d, *J* = 7.6 Hz, 1H), 7.78 (d, *J* = 15.3 Hz, 2H).

Synthesis of Compound **2** : Briefly, 4-Nitroacetophenone , 1-pyrenecarboxaldehyde and 3 M NaOH in ethanol was stirred at room temperature for 2-3 hours. The resulting solid was collected by filtration and recrystallized from ethyl acetate/acetic acid (v/v ¼ 1 : 1) to get orange crystals. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.94 (d, *J* = 15.4 Hz, 1H), 8.49 (d, *J* = 9.3 Hz, 1H), 8.36 (d, *J* = 8.1 Hz, 1H), 8.20 – 8.12 (m, 2H), 8.13 – 8.02 (m, 6H), 8.02 – 7.96 (m, 2H), 7.71 (d, *J* = 15.3 Hz, 1H), 7.47 – 7.45 (d, *J* = 8.3 Hz, 1H).

ADDITIONAL SPECTRAL DATA

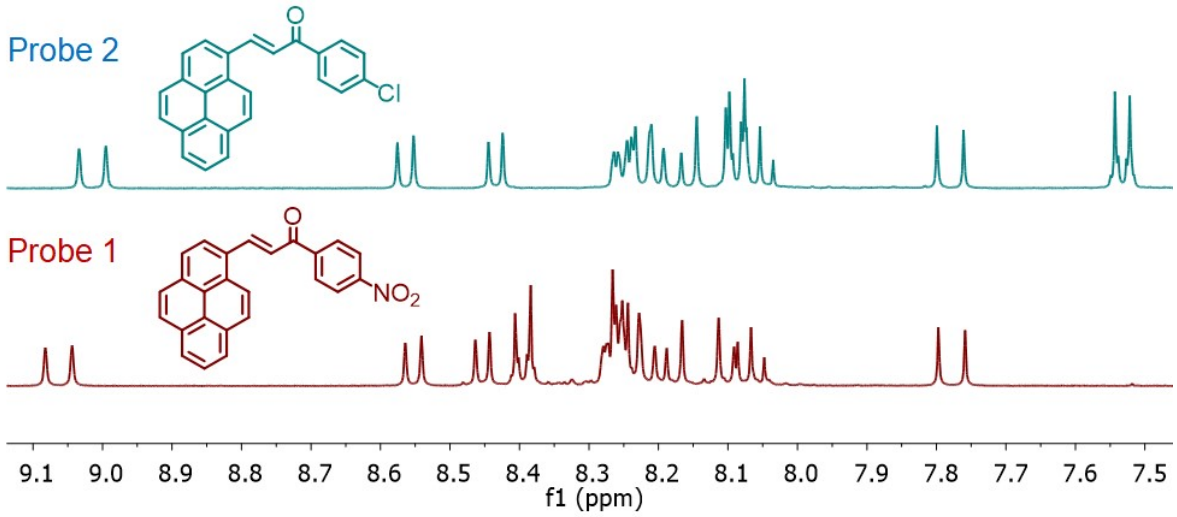


Figure. S1. Partial ^{1}H-NMR of probes **1** and **2** in $CDCl_3$

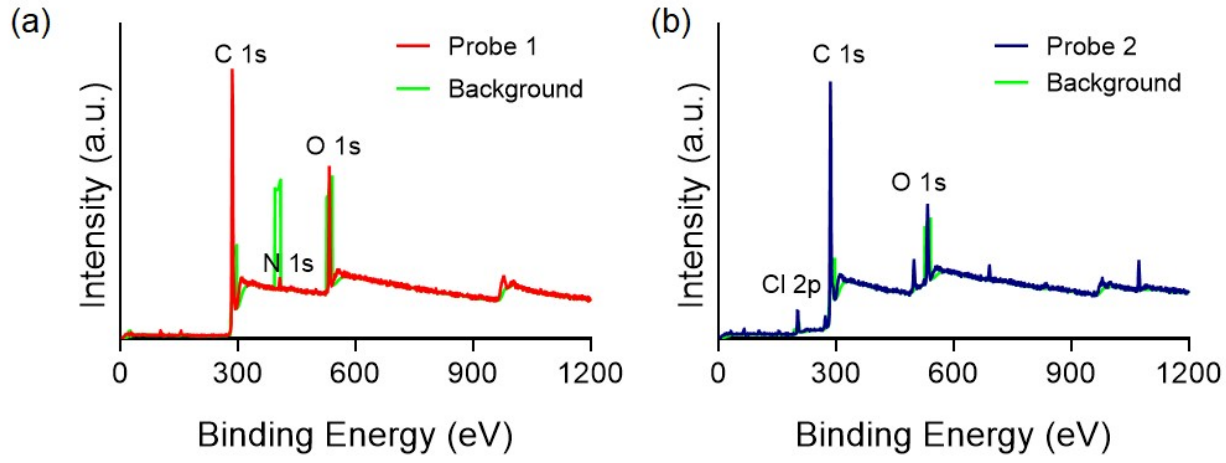


Figure. S2. XPS spectra of (a) probe **1** and (b) probe **2**.

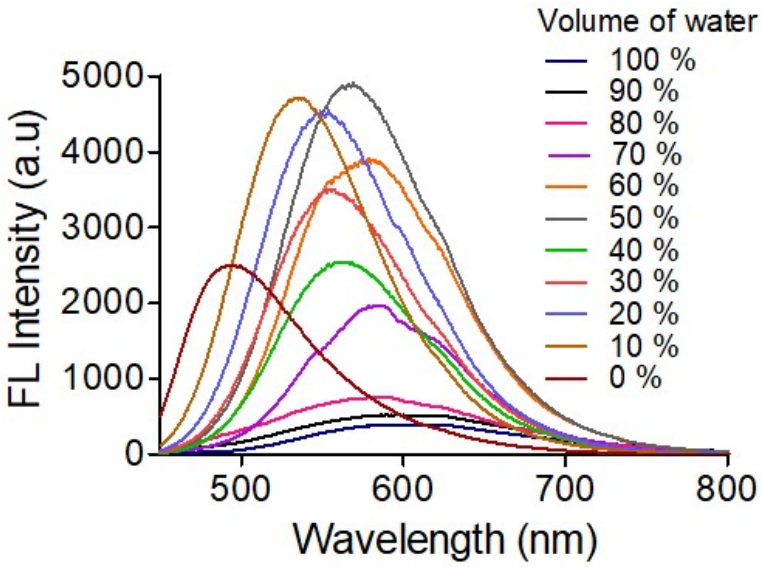


Figure. S3. Fluorescence spectra of compound **2** (10 μM, λ_{ex} = 435 nm) in different ratios of THF-water mixtures.

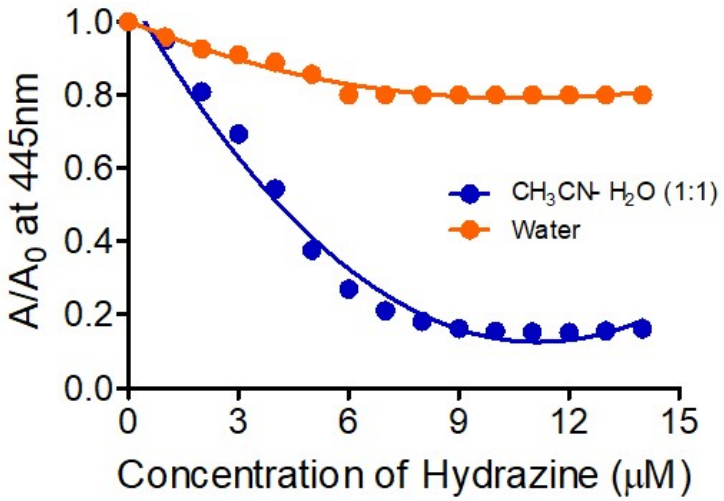


Figure. S4: UV-visible titration of **1** (10 μM) with hydrazine (0 –15 μM) in CH_3CN -Water (1:1) medium.

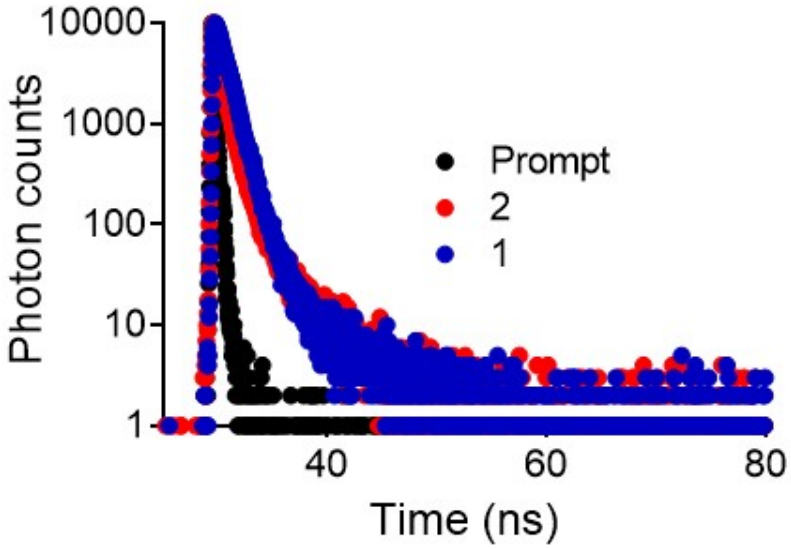


Figure. S5. Fluorescence lifetime spectra of **1** (10 μM, λ_{ex} = 435 nm) and **2** (10 μM, λ_{ex} = 435 nm) in CH_3CN-Water (1:1) at 624 nm and 585 nm respectively.

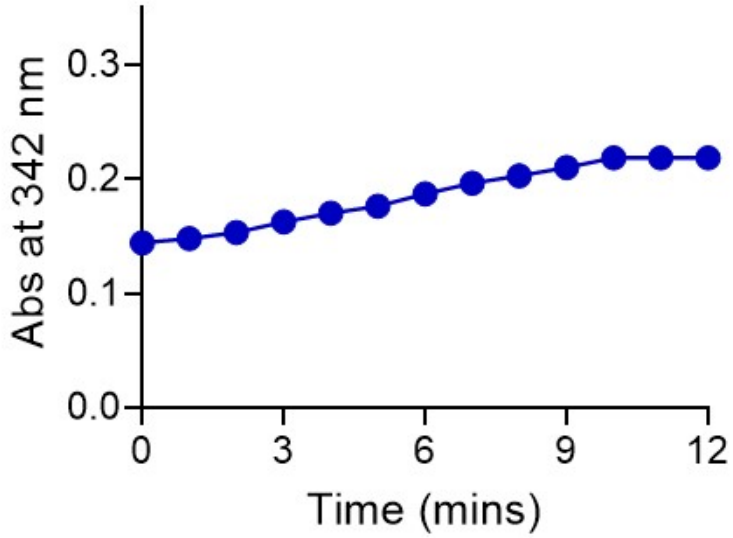


Figure. S6: Time-dependent change in absorbance of the probe at 342 nm upon addition of hydrazine (60 μM).

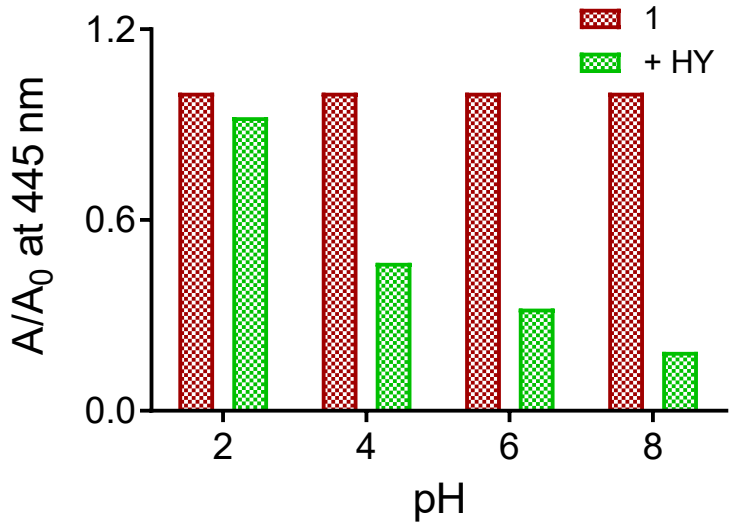


Figure. S7: Change in absorbance of **1** (10 μM) at 445 nm with hydrazine in different pH conditions.

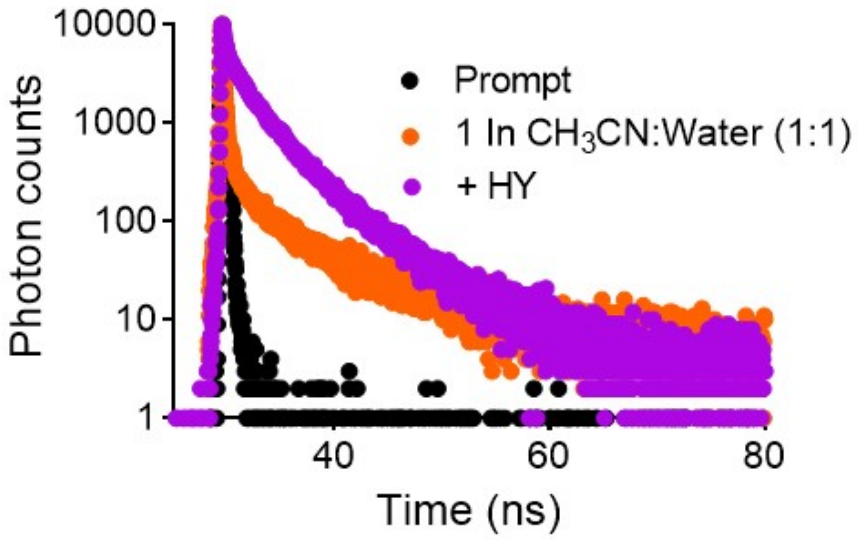


Figure. S8: Fluorescence lifetime spectra of **1** (10 μM, λ_{ex} = 435 nm) upon hydrazine addition (15 μM) in CH_3CN-Water (1:1) at 480 nm.

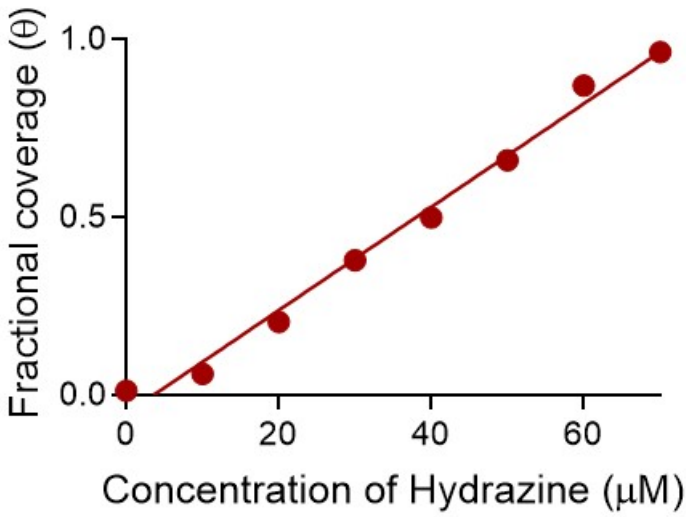


Figure. S9: Dependence of fractional coverage on incremental addition of hydrazine (0-70 μM) to probe **1** (10 μM).

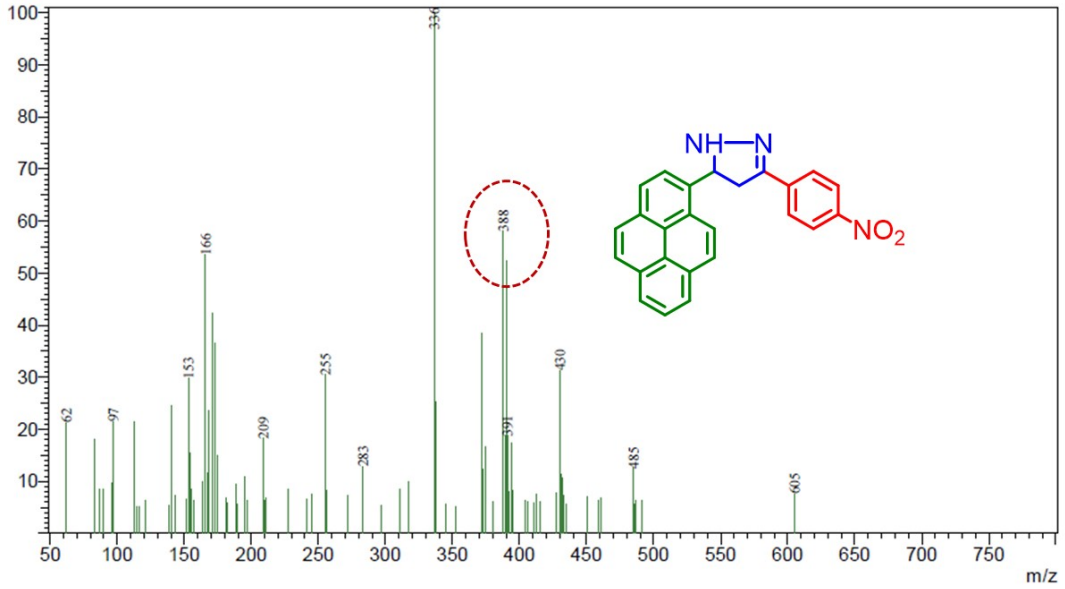


Figure. S10: Mass spectra of probe **1** upon addition of Hydrazine.

Table. S1: Fluorescence lifetime values of probes **1** and **2** in CH_3CN:Water (1:1) medium at 624 nm and 585 nm respectively.

System	α_1	α_2	α_3	τ_1(ns)	τ_2(ns)	τ_3(ns)	Average lifetime (ns)
1 in CH_3CN-Water (1:1) (at 624 nm)	0.63	0.36	0.01	0.209556	0.778043	2.97434	2.41
2 in CH_3CN-Water (1:1) (at 585 nm)	0.04	0.01	0.95	0.197529	0.967338	4.681	4.68

Table. S2: Fluorescence lifetime values of probe **1** upon hydrazine addition in CH_3CN:Water (1:1) medium at 480 nm.

System	α_1	α_2	α_3	τ_1(ns)	τ_2(ns)	τ_3(ns)	Average lifetime (ns)
1 in CH_3CN-Water (1:1) (at 480 nm)	0.0	0.0	1	1.5776	8.05504	0.0355764	3.68
1+N_2H_4 in CH_3CN-Water (1:1) (at 480 nm)	0.17	0.79	0.04	2.12216	0.121275	5.64343	3.00

Table. S3: Comparison of results obtained from the current proposed techniques with other methods.

System	Solvent medium	Method	LOD	References
Naphthalene-2,3-dialdehyde		HPLC	0.001 µM	Anal. Chem., 2012, 67, 360–363
GO/CTS/Pt		Electrochemistry	3.6 µM	Sens. Actuators, B, 2016, 236,192–200
Polyaniline (PANI3)	PANI film	Ultraviolet–visible (UV–vis)	0.49 ppm	Materials Science in Semiconductor Processing, 2018, 33, 24-31.
Nickel hexacyanoferrate	0.1 M $NaNO_3$	Amperometry	3 ppm	Russ. J. Electrochem,, 2001, 37, 1149-1153.
Polyaniline/graphene	PBS solution	Cyclic voltammetry	1.53 uM	Sens. Actuators, B, 2012, 173, 177-183.
Pyrene chalcone	Water:CH_3CN (1:1)	UV–vis Fluorescence Cyclic Voltammetry EIS [Multimodal techniques]	0.22 uM	This work

Table S4. Quantitative estimation of hydrazine (detection limit, recovery values and relative standard deviations) in different water samples

Sample	LOD (ppb)	Recovery value (%)	% RSD
Lab water	7.36	97.6 - 103.6	2.1 – 3.2
Pond water	7.55	96.8 – 104.2	2.6 - 3.7
Industrial water	7.12	95.6 - 101.6	2.2 – 3.1
Sewage water	7.6	97.3 – 102.5	2.9 - 3.6

Reference

1. Yu, F.; Wang, M.; Sun, H.; Shan, Y.; Du, M.; Khan, A.; Usman, R.; Zhang, W.; Shan, H.; Xu, C. J. R. a., Tuning the solid-state fluorescence of chalcone crystals via molecular coplanarity and J-aggregate formation. *RSC Adv* 2017, 7 , 8491-8503.