Supporting Information

Dual responsive fluorescence switching in organohydrogel towards base-acid

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EXPERIMENTAL SECTION

Materials and Physical Methods:

Phloroglucinol was purchased from spectrochem Pvt. Ltd., Mumbai. Solvents Trifluoroacetic acid, DCM, methnaol and ethanol were purchased from Sisco Resrach Laboratories Pvt. Ltd., Mumbai, India. Anhaydrous $MgSO_4$ was purchased from Himedia Laboratories, Mumbai. HCl (~11.63 M) and 25% NH_3 aqueous solution (14 M) was purchased from Molychem, Mumbai, India.

Elemental analysis was done using an Exeter CHN Analyser CE-440. FT-IR and UV Vis study of gelator, solutions and xerogel were performed on PerkinElmer- spectrum two spectrometer and UV2600 Schimazdu spectrophotometer, respectively. FESEM images were captured by JEOL-7610F Plus. ESI-MS spectra were recorded on Waters (Micro mass MS Technologies) Q-Tof Premier instrument. ^{1}H NMR spectraal study was done using a Bruker AVANCE III 400 Ascend Bruker Biospin Internation AG spectrometer and Model AVNACE NEO500 Ascend Bruker BioSpin, International AG, Switzerland. Fluorescence life time decay data was measured using a HORIBA Jobin Yvon picosecond time-correlated single photon counting (TCSPC) spectrometer (model Fluorocube-01-NL).

Confocal Laser Scanning Microscopy (CLSM): Confocal microscopic images were obtained for the freshly prepared **OHG** gel on a OLYMPUS (model-IX-83). 20 μL aliquots of diluted **OHG** (DMSO-water mix solvent) was then drop-casted on the coverslip. The images were recorded thereafter in the gel phase. The excitation laser of 405 nm and detection wavelength regions of 550-650 nm were employed.

Rheological Study:

The mechanical measurements were performed using a stress-controlled Rheometer (Anton Paar TWINE Rheometer MCR 700) equipped with stainless steel parallel plates (20 mm diameter, 1.0 mm gap). Rheological experiments were carried out on freshly prepared organogel (0.35 % w/v). Linear viscoelastic regions (LVR) of the organogel was determined by measuring the storage modulus, G′ (associated with energy storage) and the loss modulus, G″ (associated with the loss of energy) as a function of stress amplitude (Dynamic oscillatory frequency of 1 rad s^{-1}). The following tests were performed: increasing amplitude of oscillation up to 100 % apparent strain on shear, time and frequency sweeps at 20 °C (20 min and from 0.1 to 100 rad s^{-1}, respectively). All these measurements were conducted in triplicate.

Synthesis and characterization:

H_3BTA or 2,4,6-trihydroxybenzene-1,3,5-tricarbaldehyde

10 g (71.5 mmol) of Hexamethylene tetramine (HMTA) was first dissolved in 40 mL of TFA (trifluoroacetic acid) and added dropwise to the ice cooled 20 mL suspension of phloroglucinol (3.98 g; 31.5 mmol) in TFA. The resulting mixture was then kept under reflux for 5 hrs. Further, 150 mL of 3M HCl was added to the obtained orange solution and put under reflux for another 2 hrs. The resulting solution was then extracted using 150 mL of DCM thrice and dried over anhydrous $MgSO_4$.[1] The solution was filtered and dried in vaccuum dessicator to obtain a yellowish orange fine powder. Yield 1.4 g (16%). ^{1}H NMR (*d_6*-DMSO, 400 MHz, δ_H, ppm) 14.04 (s, 3H, -OH), 10.08 (s, 3H, -CHO).

5,5',5"-((1E, 1'E, 1"E)-((2,4,6-trihydroxybenzene-1,3,5-triyltris(methanylylidene)) tris(azanylylidene))triisophthaic acid or TH-AIL

H_3BTA (0.300 g, 1.43 mmol) dissolved in chloroform was added dropwise to the methanolic solution of 5-aminoisophthalic acid (0.775 g, 4.28 mmol) at room temperature. The resulting solution kept under constant stirring for another 6 hrs. The orange coloured precipitate **TH-AIL** was then filtered and quick washed with cold methanol twice and dried in vaccuum dessicator. Yield 0.700 g (70%). Anal. calcd for $C_{33}H_{21}N_3O_{15}$: C, 56.66; H, 3.03; N, 6.01. Found C, 56.71; H, 3.08; N, 5.96. ^{1}H NMR (*d_6*-DMSO, 500 MHz, δ_H, ppm) 7.90-8.31 (9H, Ar), 8.57 (s, 3H, -CH=N), and 13.15 + 12.85 (d+q, 6H, -COOH). ^{13}C NMR (*d_6*-DMSO, 500 MHz, δ_H, ppm) 184.57, 166.23, 139.82, 133.13, 126.58, 121.99, 107.34. ESI-MS: $[M+H]^+$, *m/z*, 700.09 (*calcd*. 700.09).

FT-IR (cm^{-1}) $\upsilon(C=O)_{strech}$ 1711 and $\upsilon(C=N)_{strech}$ 1584. UV-visible spectrum (DMSO, $2x10^{-5}$M) [λ, nm (ε, M^{-1} cm^{-1})]: 321 (6800), and 400 (13250).

Synthesis of OHG organohydrogel:

In a 5 mL glass vial, 3.5 mg TH-AIL was dissolved in 500 μL of DMSO solvent with the help of mild sonication which yielded orange coloured solution. To this gelator solution, 500 μL deionized water was added resulting in instantaneus bright orange coloured organohydrogel **OHG** formation at room temperature. The gel for mation was priliminary confirmed by inverted vial test method.

Experimemental details of base and acid responses by OHG:

OHG was prepared at its minimum gelation concentration (0.5×10^{-2} M) and the 6 equivalents of aq. NH_3 solution was required to completely quench the orange fluorescence. For the experiment depicted in S13 and S14, $NH_3.H_2O$ was taken as aqueous solution (0.5 M, prepared from as-obtained 25% $NH_3.H_2O$) solution where a total of 6 equivalent NH_3 was added stepwise with an equal step size of 1 equivalent. Notably, as evident from Table S2 and Figure S6, increment of water in **OHG** system does not disturb gelation property as the gel can form at even higher water content (e.g., 40:60). Hence, the base and acid analyte solutions were prepared as pure aqueous solutions. Moreover, to avoid much dilution during analyte addition, base or acid concentrations were always kept 2 fold higher than the diluted **OHG** concentration. For the other bases *viz.*, diethylamine, ethylenediamine, triethyl amine, hydrazine identical conditions were maintained. Further, to regain the fluorescence and gel phase, the acids $HCl/HClO_4/HNO_3/H_2SO_4$ (0.5 M) in their aquous solution phase were exposed to above NH_3 containing fluorescence quenched solution. As observed, after neutralisation of NH_3 in **OHG** solution by HCl solution, additional 2 equivalents HCl was required to retrieve the fluorescence of the **OHG** gel. Hence a total of 8 equivalents of HCl was needed to reattain the emission as well as gel phase.

Method for Vapor phase response of OHG gel towards NH_3-HCl:

A 5 mL vial with 2 mL **OHG** gel (0.5×10^{-2} M **TH-AIL**) was placed in a 100 mL beaker containing 1 mL of ammonium hydroxide solution. The set up was then sealed and kept at room temperature. The orange fluorescent gel turned into weakly green fluorescing

solution within 2 mins as the ammonia diffused into the system. The process was observed under the UV- lamp of 365 nm. To regain the gel phase **OHG**, this solution first kept in open air for excess ammonia evaporation and then placed in a 100 mL beaker containing 3 mL of HCl. The solution turned into gel within 10 minutes with the restoration of fluorescence properties.

The method adopted for gas phase NH_3/HCl responsive behaviour of **OHG** has been demonstrated in figure S16. The gas phase treatment by NH_3 and HCl were conducted by using standard 25% ammonium hydroxide solution (14 M) and standard HCl solution (11.63 M) in a closed beaker. As described above, the **OHG** gel (0.5 x 10^{-2} M **TH-AIL**) was gently placed in the same closed beaker. Ammonium hydroxide (20 °C, 25%, 0.907 g mL^{-1}) was used as a gaseous resource for NH_3. The concentration of ammonia was calculated to be by following the formula-

$$C_{ammo} = \frac{0.907\ x\ 0.25x\ Vol_{NH_3.H_2O}}{Vol_{chamber}}$$

$Vol_{NH_3.H_2O}$ is the volume of ammnium hydroxide taken in the closed chamber, $Vol_{chamber}$ is the volume of the chamber taken.

In case of HCl solution, the HCl fume was the source of HCl.

Limit of Detection determination and association constant determination:

To calculate the sensibility limitation for NH_3, 10^{-2} M aq. ammonium hydroxide solution was added in stepwise manner from 0 mM to 1.6 mM to the 2.5 mL of diluted **OHG** (**(**10^{-4} M, DMSO:water = 1:1 mix solvent). The results revealed that the fluorescence of **OHG** at 582 nm quenched step wise with gradual increase of the concentration of ammonia (Fig. S11). The fluorescence completely quenched as the concentration of ammonia was increased to 1.6 mM. To obtain the slope of the curve, the change in fluorescence intensity at 582 nm was plotted against variable NH_3 concentration as shown in figure S11. The calibration curve of fluorescence intensity *vs* ammonia concentration was found linear with a correlation coefficient (R^2) of 0.99612. The limit of detection was determined to be 0.177 μM (Table S3).

To determine the LOD for HCl, diluted **OHG** was first quenched by addition of 5 equivalents of NH_3 using 10^{-2} M ammonium hydroxide aqueous solution and the fluorescence intensity was regained by using 10^{-1} M HCl aqueous solution so as to

prevent excess dilution the LOD experiment (Figure S12). The calibration curve of fluorescence intensity *vs* HCl concentration was found to be linear with a correlation coefficient (R^2) of 0.98529. The limit of detection was determined to be 1.58 µM (Table S3).

The nature of the binding interaction of **OHG** to the NH_3 and HCl was evaluated using the Benesi–Hildebrand equation from the fluorescence titration of diluted **OHG** solution (1 x 10^{-5} M, DMSO:water = 1:1 mix solvent) against NH_3 (1 x 10^{-3} M, aq.) and HCl (1 x 10^{-3} M, aq.) solutions in consecutive steps (Fig. S13, S14, ESI†).[2-4] The slope and intercept of the linear plot obtained from the double-reciprocal plot between $1/[I_0\text{-}I]$ *vs.* $1/[NH_3]$ and $1/[I\text{-}I_0]$ *vs.*$1/[HCl]$ for NH_3 and HCl response in the gel gave rise to the association constants, respectively.

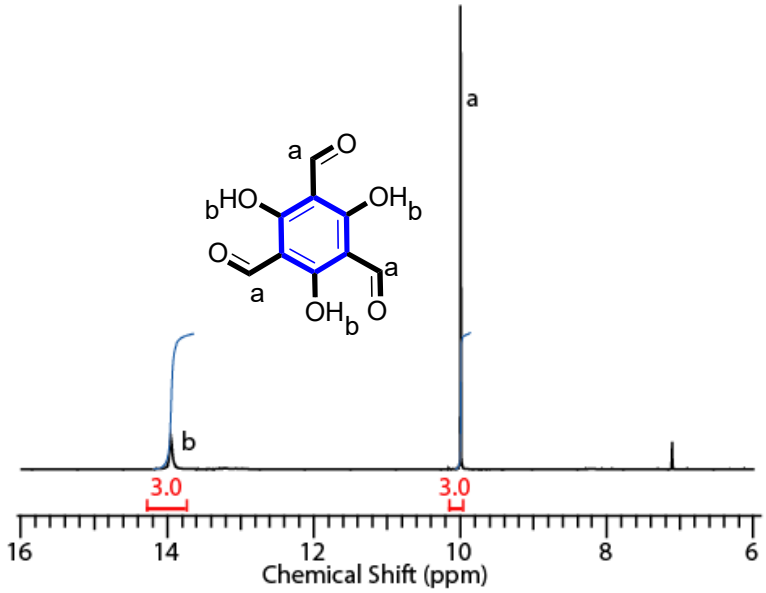
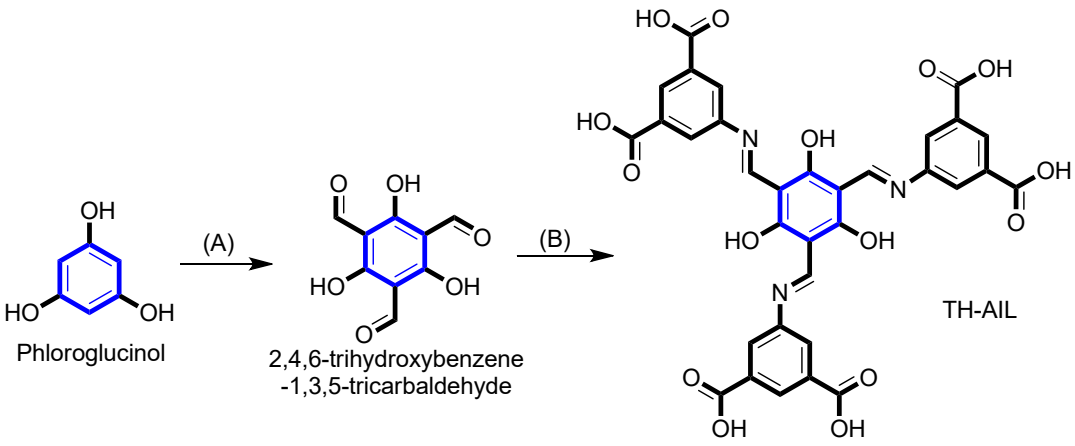


Figure S1. ^{1}H NMR of **H_3BTA** ($CDCl_3$) along with peak positions and its labelled chemical structure.



Sc (A) HMTA, TFA, 60°C, 2h (B) 5-aminoisophthalic acid, MeOH, RT, 6h

heme S1. Two step synthetic process for chemical synthesis of **TH-AIL**.

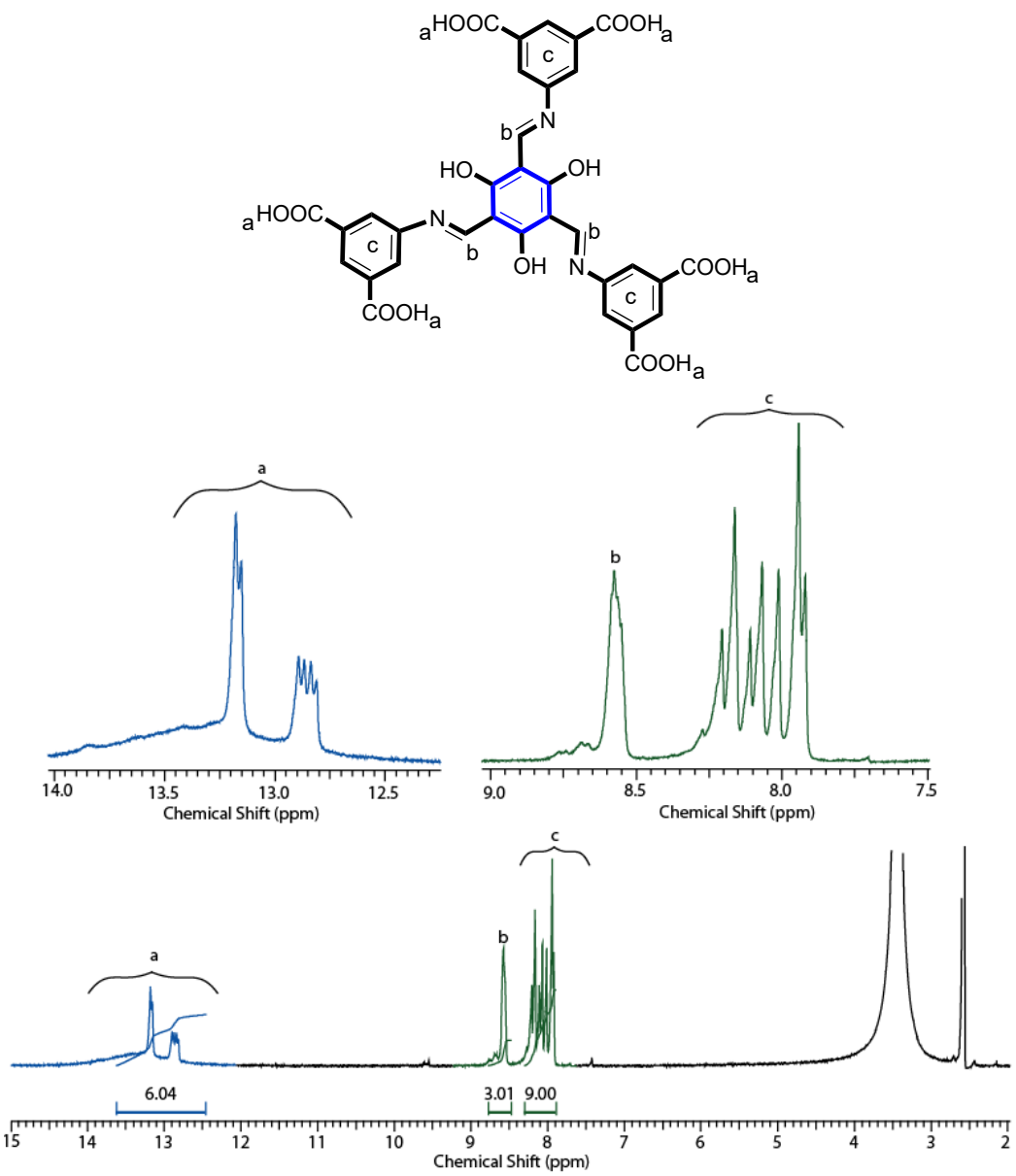


Figure S2. ^{1}H NMR spectra (d_6-DMSO, 500 MHz, δ_H, ppm) for **TH-AIL** along with the labelled chemical structure.

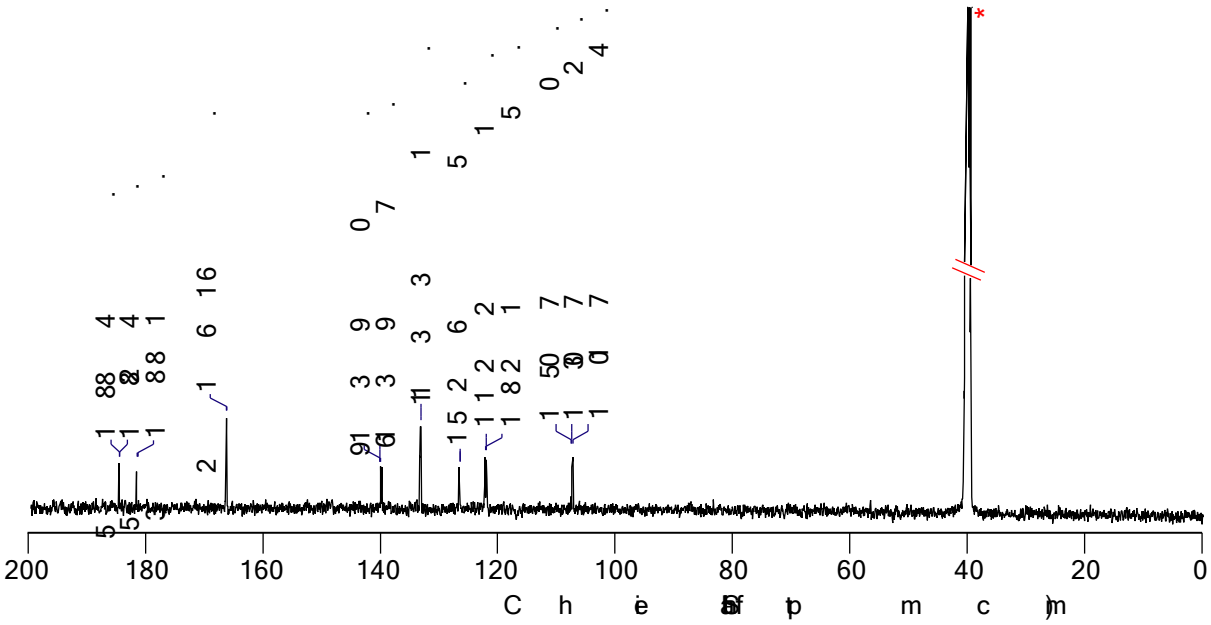


Figure S3. ^{13}C NMR spectra (d_6-DMSO, 500 MHz, δ^{13}C, ppm) for **TH-AIL**.

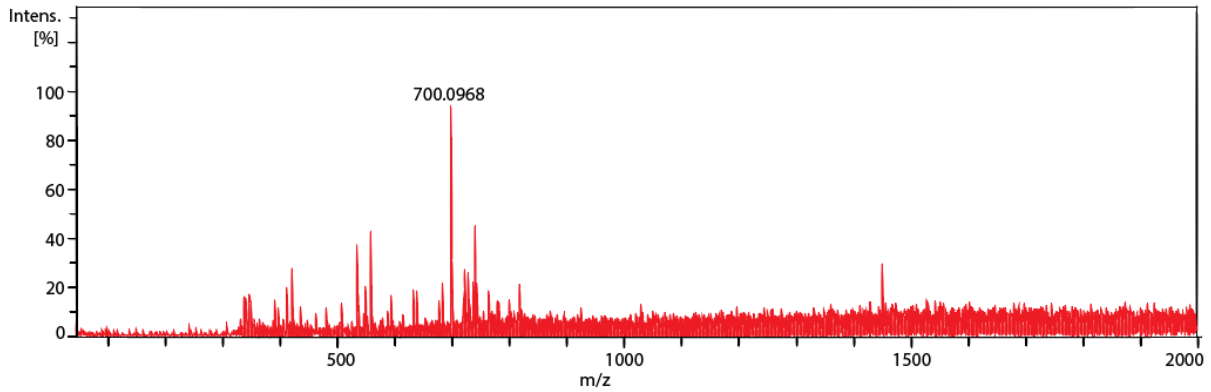


Figure S4. ESI-mass spectra of synthesized **TH-AIL**.

Table S1. Gelation tests with respect to gelator TH-AIL and solvents*

S.N.	Solvent	TH-AIL
1.	Water	P
2.	Acetonitrile	P
3.	Methanol	P
4.	Ethanol	P
5.	DMF	P
6.	DMSO	S
7.	DMSO+Water	**G**

*Where, S= solution, P= precipitate, G= gel.

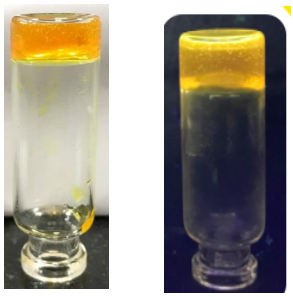


Figure S5: Image of **OHG** gel under (i) visible light, (ii) under UV light.

Table S2: Gelation trial at different DMSO:water ratios (v/v, where total volume was kept 1 ml) where **TH-AIL** (0.35 wt% or 3.5 mg) was dissolved in DMSO and water was added afterwards.*

DMSO: Water ratio (v/v)	**Gelation (0.35 wt% TH-AIL)**
2:8	Solubility in DMSO was not appreciable
3:7	PG
4:6	G
5:5	G

*Where, PG= Partial Gel; G= Gel.

Figure S6: Gelation optimisation at different DMSO: Water ratios (a) 2:8; (b) 3:7; (c) 4:6; (d) 5:5.

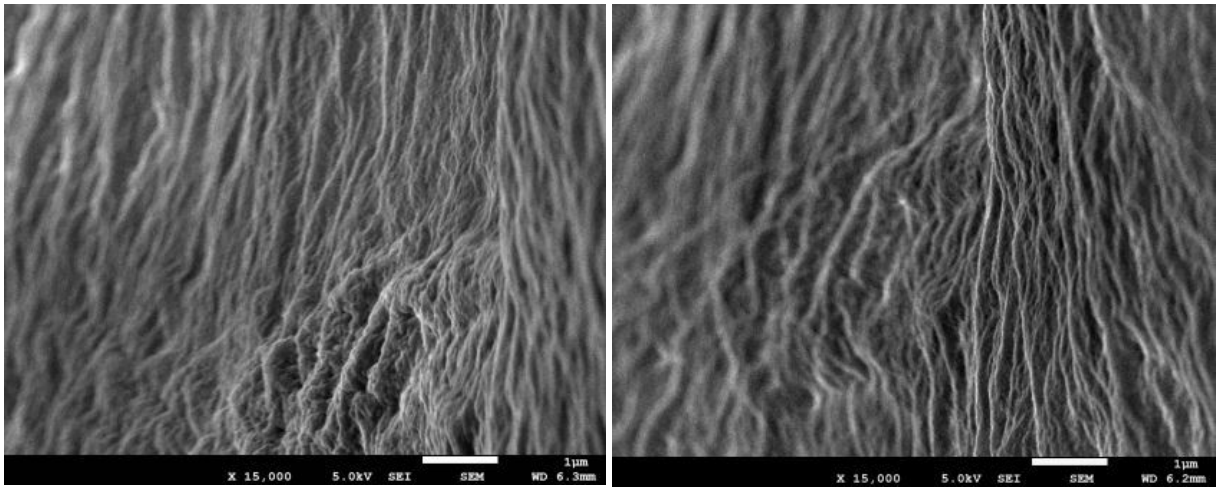


Figure S7. FESEM images of dried **OHG** organohydrogel.

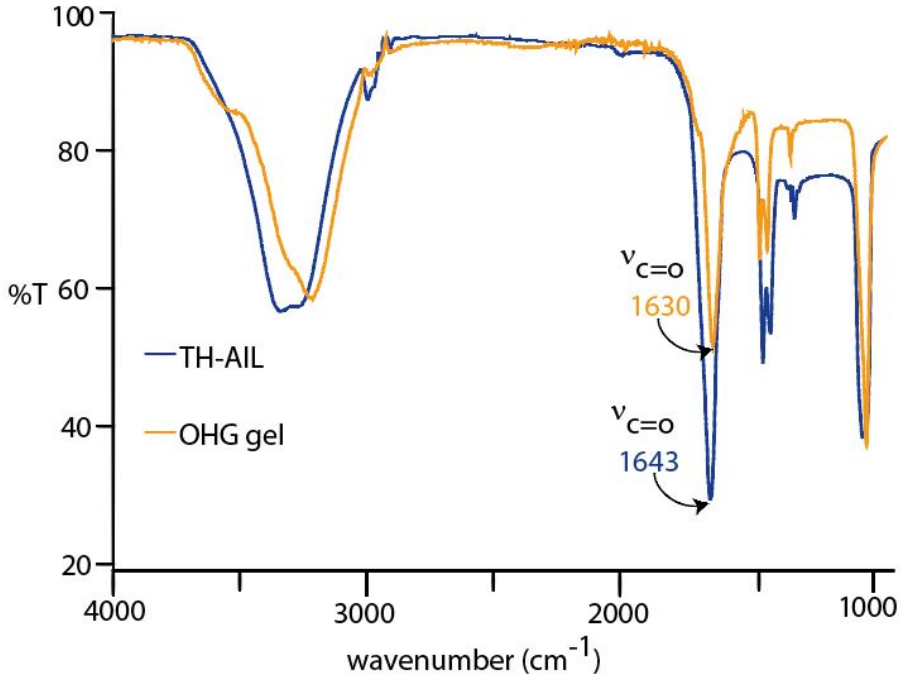


Figure S8. FTIR for **TH-AIL** (blue line) and **OHG** (orange line).

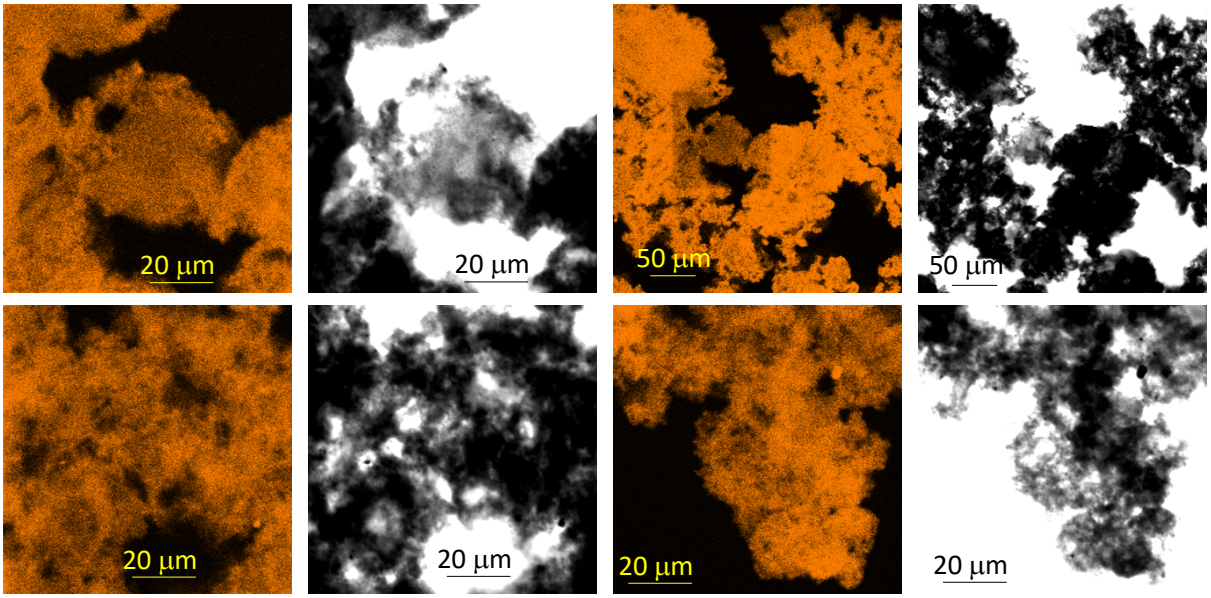


Figure S9. Confocal Laser Scanning Microscopic (CLSM) images of **OHG** at two different magnifications of 50 and 20 µm, (excited using 405 nm laser).

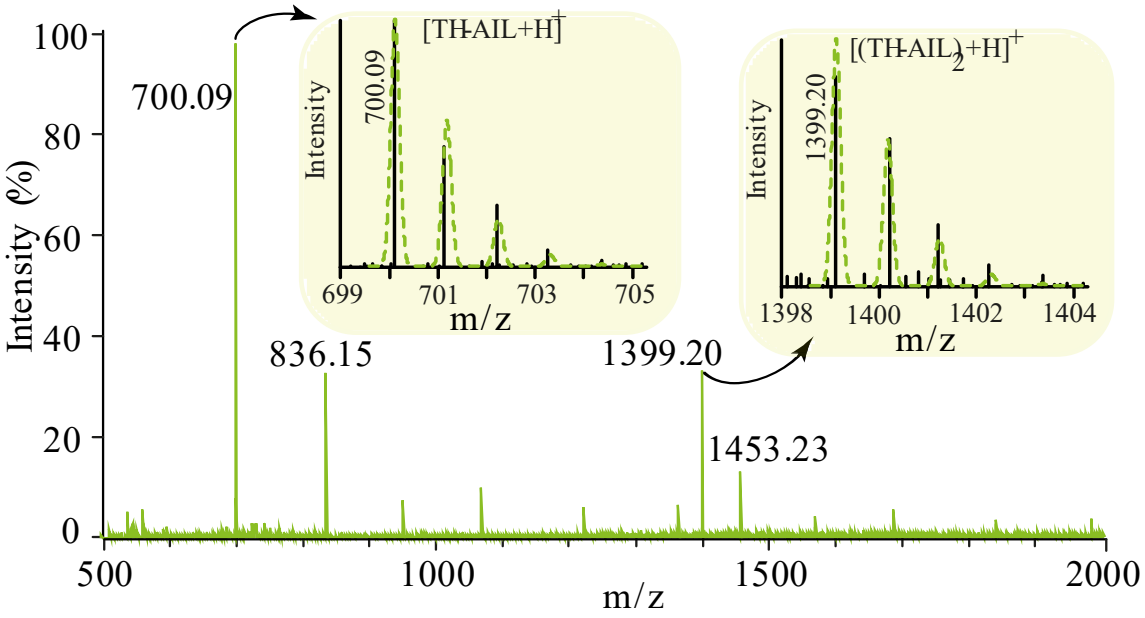


Figure S10. ESI-mass spectrum of diluted **OHG** (10^{-3} M).

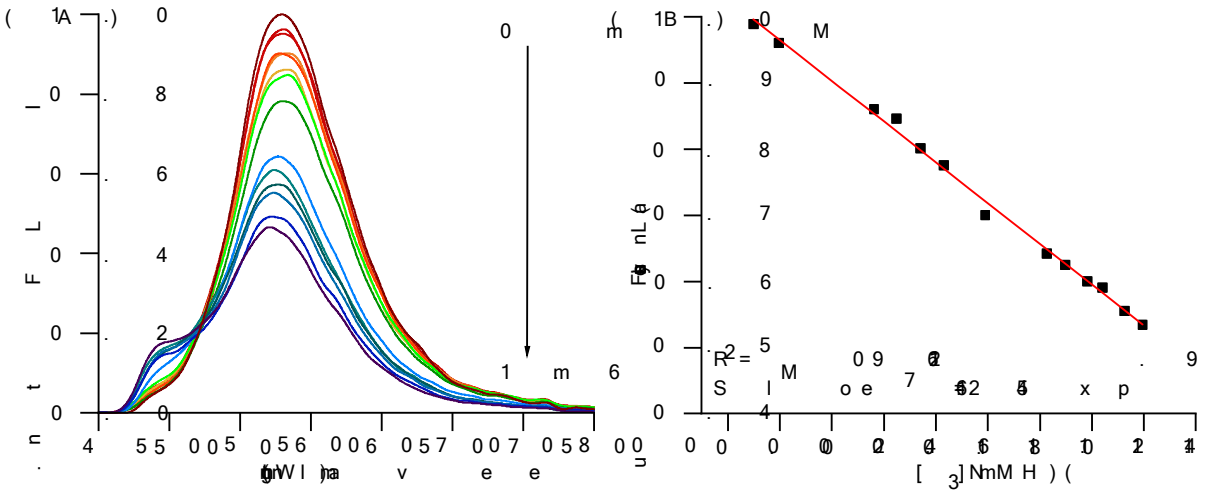


Figure S11: (A) Fluorescence spectra of diluted **OHG** (10^{-4} M, diluted with 1:1 DMSO/water, dark brown line) with increasing concentration of NH_3 (10^{-2} M, aq.). SD was calculated as 0.9608454661, (B) Linear plot for the NH_3 concentration-intensity correlation, emission change was observed at λ=570 nm.

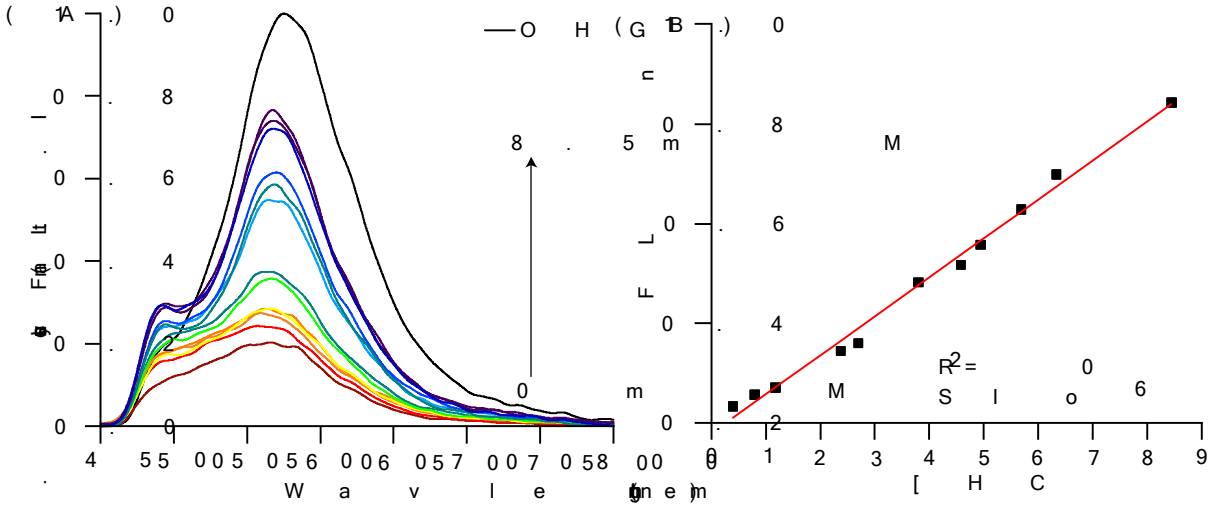


Figure S12: (A) Fluorescence spectra of diluted **OHG** (10^{-4} M, black line, DMSO:water = 1:1) quenched with NH_3 (10^{-2} M, dark brown line, aq.) treated with HCl (10^{-1} M, aq) in the stepswise manner. SD was calculated as 0.934118; (B) Linear plot for the HCl concentration-intensity correlation, emission change was observed at λ=565 nm.

Table S3: Limit of detection (LOD) data for analytes NH_3 and HCl derived from figure S11 and S12 -

Analyte	Slope	SD	R^2	LOD
NH_3 (aq.)	-1.6254×10^7	0.960845461	0.99612	0.177 µM
HCl	1.764×10^6	0.934118	0.99199	1.58 µM

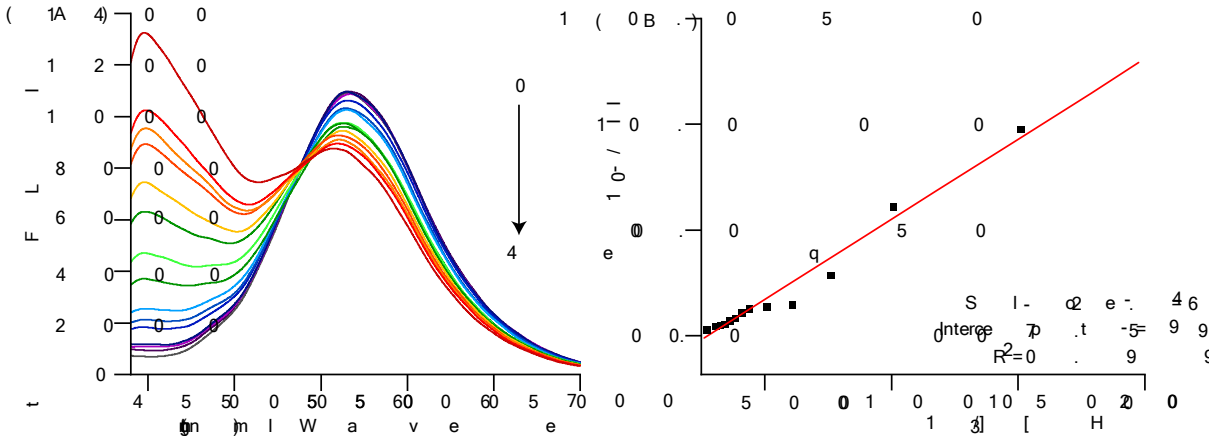


Figure S13: (A) Fluorescence titration of diluted **OHG** (1 x 10^{-5} M, solvent DMSO/water=1:1) with increasing NH_3 (10^{-3} M, water solvent) and corresponding (B) Benesi–Hildebrand linear plot, where emission change was observed at 570 nm.

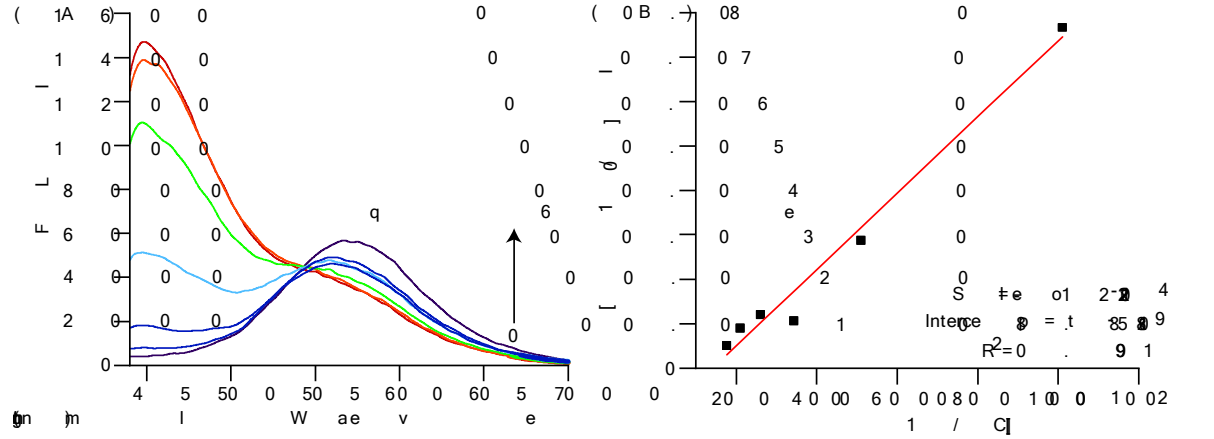


Figure S14: (A) Fluorescence titration of diluted **OHG-NH_3** (~1 x 10^{-5} M, DMSO/water =1:1, 5 eqv NH_3 was added, aq.) adduct (red line) with increasing HCl (10^{-3} M, aqueous) and corresponding (B) Benesi–Hildebrand linear plot, where emission change was observed at 565 nm.

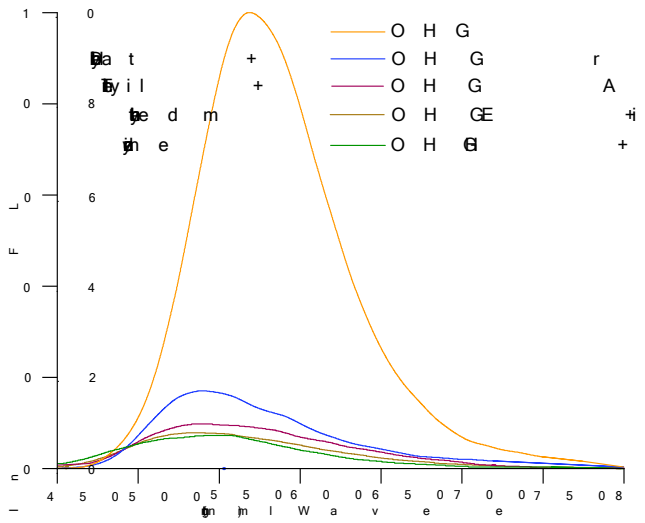


Figure S15. Fluorescence spectrum of **OHG** (0.5 x 10^{-2} M, DMSO/water = 1:1; λ_{ex}= 420 nm; orange line) with 0.5 M aquous solutions of diethylamine, ethylenediamine, triethyl amine and hydrazine.

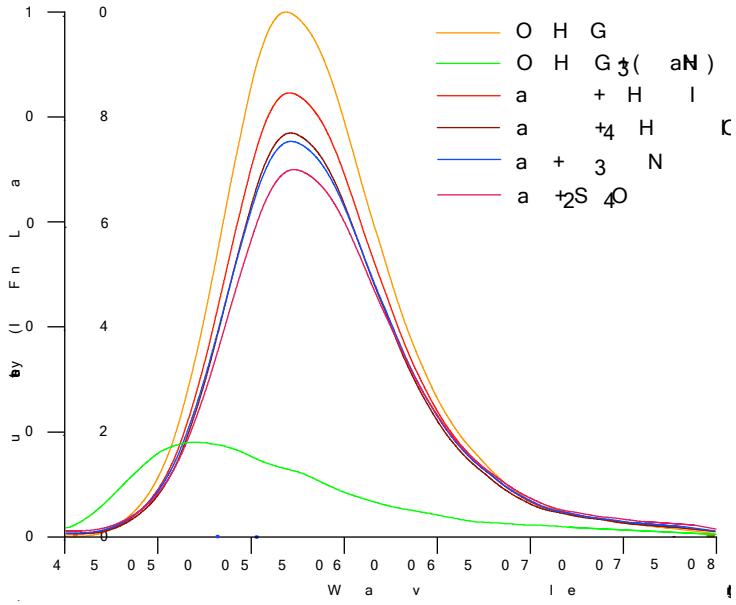


Figure S16. Fluorescence spectrum of **OHG** (0.5 x10^{-2} M, DMSO/water= 1:1; λ_{ex}= 420 nm; orange line) with ammonia (green line) and simutaneous contact of 0.5 M aquous solutions of HCl, $HClO_4$, HNO_3 and H_2SO_4.

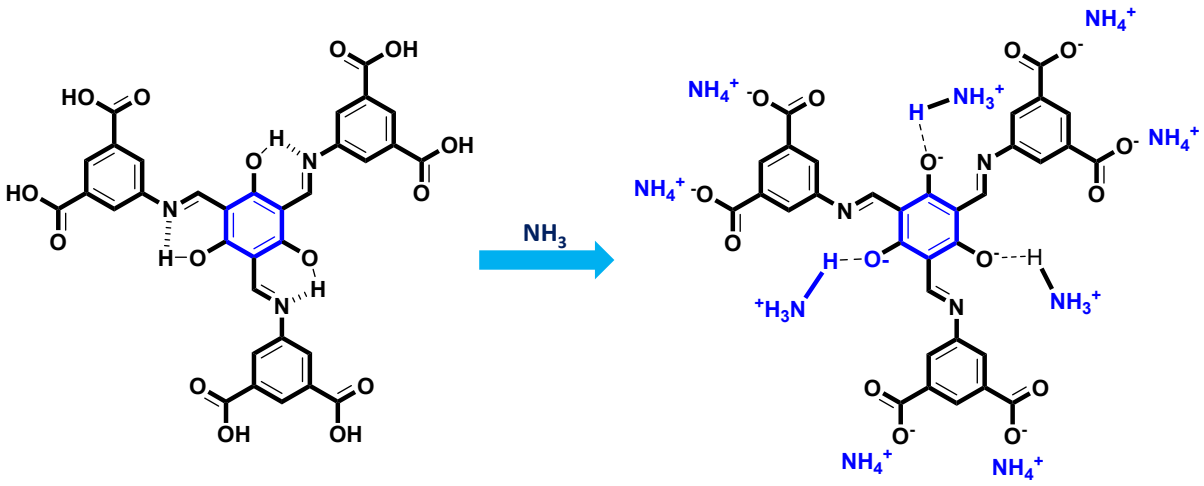


Figure S17. Plausible mechanism of NH_3 triggered gel-sol transition in **OHG** *via* **TH-AIL-**NH_4^+ adduct formation.

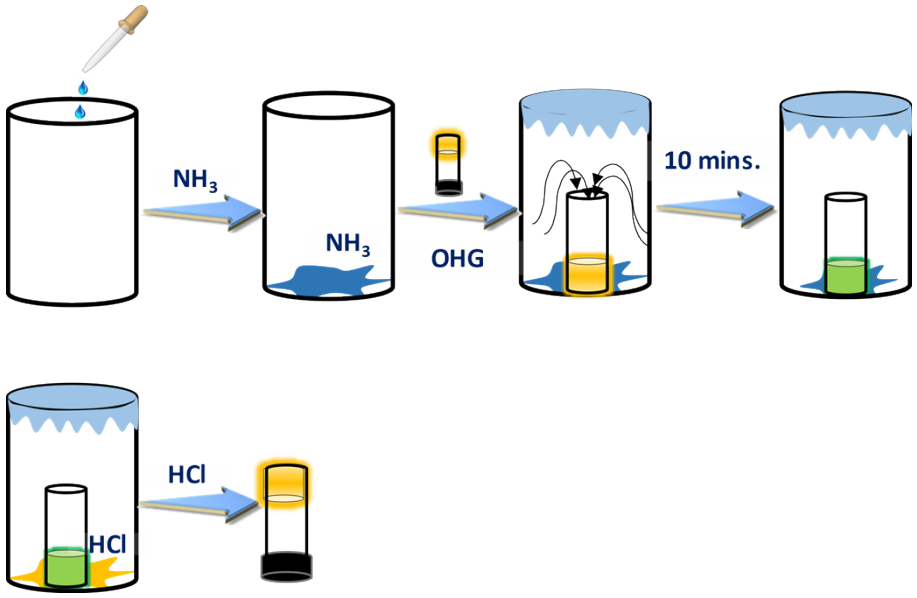


Figure S18. Schematic demostration of process adopted for the vapor phase response of **OHG** gel towards NH_3-HCl.

Note: A 5 mL vial with 2 mL **OHG** gel (0.5 x 10^{-2} M **TH-AIL, DMSO/water** =1:1) was placed in a 100 mL beaker containing 1 mL of NH_3. The set up was then sealed and kept at room temperature. The gas phase treatment by NH_3 and HCl were conducted by using standard 25% ammonium hydroxide solution (14 M) and standard HCl solution (11.63 M) in a closed beaker.

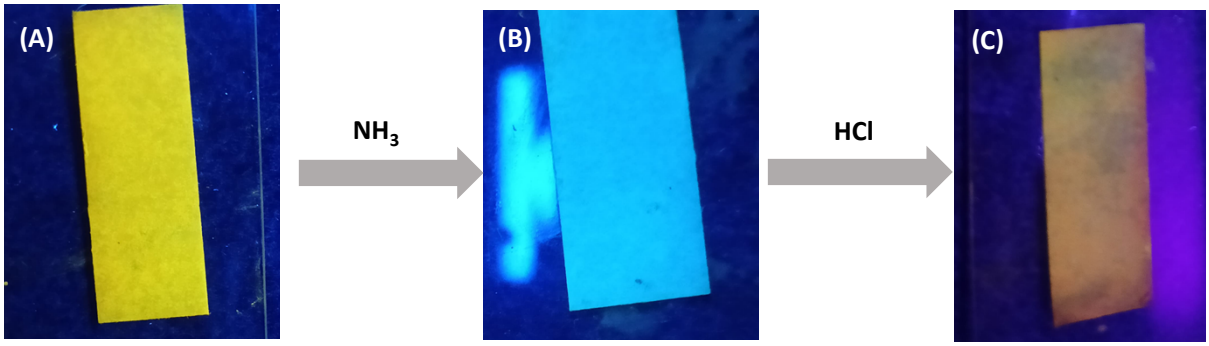


Figure S19: Dual base/acid response in a paper strip containing **OHG** xerogel.

Note: Although the strip containing xerogel immediately changed colour from bright yellow to pale blue-green upon exposure to ammonia, but the reversibility response upon addition of acid showed poor and obtained fade yellow-orange. This observation signifies the importance of gel phase reversible responsive behaviour of **OHG** gel.

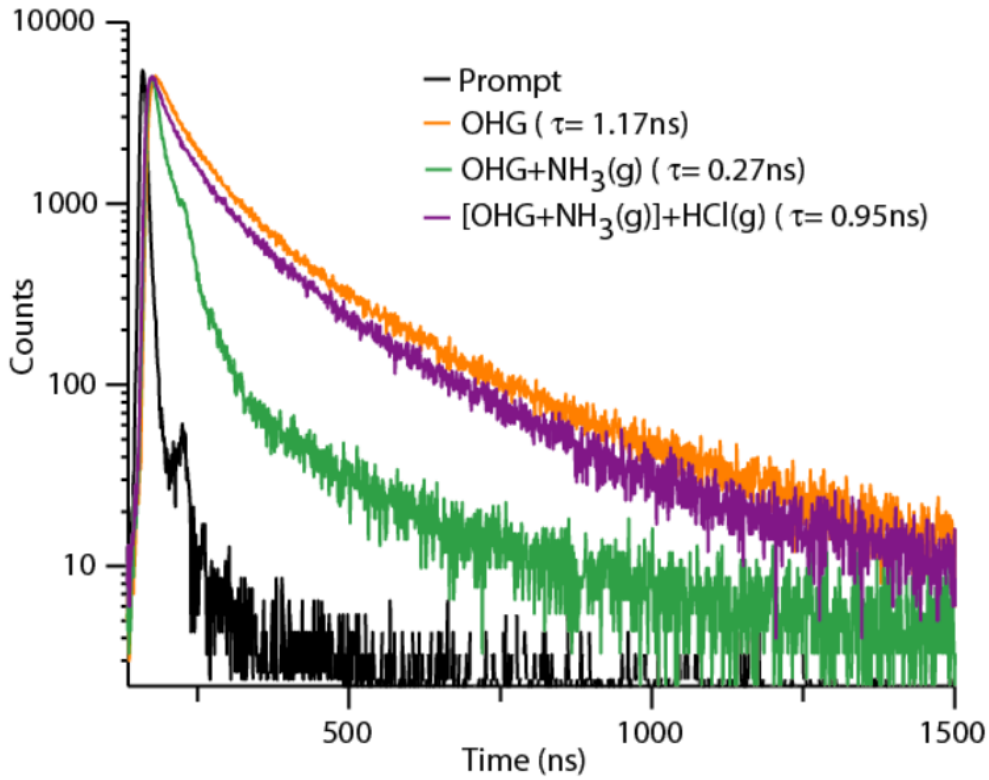


Figure S20. Fluorescence lifetime measurement plot for **OHG** (orange line), **OHG** + NH_3 (green line), [**OHG**+NH_3]+HCl (violet line) along with respective average lifetime values.

Reference:

1. J. H. Chong, M. Sauer, B. O. Patrick and M. J. MacLachlan, *Org. Lett,.* 2003, **5**, 21, 3823–3826.
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