

Supporting Information

Water soluble AIE-gen for organic-solvent-free detection and wash-free imaging of Al³⁺ ions and subsequent sensing of F⁻ ions and DNA tracking

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1. Materials and methods

a. Chemicals and reagents

4-Hydroxybenzophenone was purchased from Sigma Aldrich (India). TiCl_4 was purchased from Spectrochem Pvt. Ltd. Mumbai (India). All other common chemicals and solvents of AR grade were obtained from different commercial suppliers and were used without further purification. All ultrapure water used was collected from Millipore water system and purged with N_2 for 15 min prior to use.

b. Instruments, measurements and methods

NMR spectra were recorded on Bruker Avance (400 MHz) NMR spectrometer. HRMS spectra were recorded on Q-TOF LC-MS (6545 Q-TOF LC-MS, Agilent) using ESI as the ion source. Fluorescence spectra were taken on a JASCO FP-6300 spectrofluorimeter; the slit width was 2.5 nm for both excitation and emission. For fluorescence measurements the excitation wavelength was set to 345 nm and emission spectra were recorded in the range of 346 to 650 nm. Field emission scanning electron microscopy (FESEM) images were recorded on a Quanta 250 FEG (FEI make). Particulate Systems NanoPlus zeta/nano particle analyzer was used for DLS study. Optical micrographs were taken on inverted microscope, IX-51 (Olympus) with fluorescence attachment. THF was dried over sodium and freshly distilled before use. The reactions were monitored by thin layer chromatography (TLC) carried out on 0.25 mm silica gel plates (60F-254) using UV light (254 or 365 nm) for visualization.

c. Procedure and sample preparation for fluorimetric studies

1 mM stock solutions of **TPE-diBuS** and $\text{Al}(\text{NO}_3)_3$ were prepared in de-ionized water (MilliQ, 18 M Ω). For study of the effect of different metal ions corresponding nitrate or chloride salts were used and for anions corresponding sodium or potassium salts were used, and 1 mM of stock solutions were prepared by dissolving them in deionized water (MilliQ, 18 M Ω). The solutions of real samples were prepared by spiking $\text{Al}(\text{NO}_3)_3$ in water samples collected from various water bodies. Stock solutions of ctDNA (1 mM) were prepared in HEPES buffer. Deionized water (Milli-Q, 18 M Ω) was used as per requirements for dilution purpose. All solutions were subjected to filtration through 0.22 μm syringe filter to avoid any interference by any particulate matter in fluorescence measurement. All the experiments were carried out at room temperature (25 °C) and 5 minutes of incubation time is given between two fluorimetric measurements.

d. DLS analysis

For DLS analysis aqueous solution of **TPE-diBuS** (20 μM) was prepared and its particle size was obtained, and the same solution was treated with Al^{3+} ions (1.2 equiv) and the particle size of this solution was again measured after 15 min.

e. SEM analysis

The sample solutions drop casted on silicon wafer and coated by gold-palladium mixture at 5 nm thickness using Leica EM ACE200. The images of cells were obtained using a FEI Quanta FEG 250 scanning electron microscope.

f. DFT studies

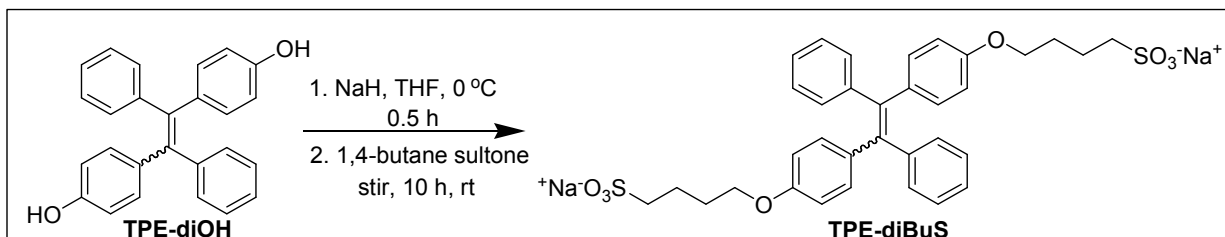
All the structural optimizations of **TPE-diBuS** and its complex with Al^{3+} were performed with Gaussian G09W using Density Functional Theory (DFT) method.¹ All the calculations were performed using functional B3LYP basis sets 6-31+G* for C, H, O, S and LANL2DZ for Al^{3+} and other metal ions.

g. Cytotoxicity and cell imaging studies

For cytotoxicity studies and imaging of HK cells (Human Kidney cells), the cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% (v/v) fetal bovine serum. 1 mM of stock solution of the probe was prepared in DMEM. 5×10^4 cells/well viable HK cells were plated in a 24-well plate and incubated at 37 °C in a humidified 5% CO_2 incubator to achieve 80% confluency. After which the probe solution was added into the culture media present in the wells of the plate with each well containing 80% confluent cells to achieve final concentrations of the probe in the media as 1 μM , 10 μM , 30 μM , 50 μM , 70 μM and 90 μM and they were incubated for another 24 h. Cytotoxicity was analysed by the MTT assay. The cell viability was expressed as % of viable-cells relative to untreated controls. Each set of experiments was triplicated and the average results are presented.

For cell imaging, HK Cells from overnight grown culture were inoculated with secondary culture to harvest the young grown cells. The grown HK cells were harvested by centrifugation at 4 °C and washed twice with sterilized HEPES solution. The washed cells were suspended in an appropriate volume of sterile HEPES. Different cell sets were prepared and loaded with different concentrations of $\text{Al}(\text{NO}_3)_3$ (1 μM , 5 μM , 10 μM , respectively) at 37 °C for 1 h. After removal of free Al^{3+} by washing the cells with HEPES buffer, **TPE-diBuS** (10 μM) respectively, incubated for another 1 h. The fluorescence and bright-field images were taken directly under an Olympus IX51 inverted microscope, combining the phase-contrast system and the fluorescence system, without further washing steps.

2. Synthesis of sodium 4-(4-(1,2,2-triphenylvinyl)-phenoxy)butane-2-sulfonate (TPE-diBuS)²



The starting TPE derivative for the probe molecule, (*E/Z*)-1,2-bis-(4-hydroxyphenyl)-1,2-diphenylethylene (**TPE-diOH**) was synthesized by McMurry coupling following the procedure reported by us.³

In a 25 mL round-bottom flask, NaH (88 mg, 1.1 mmol, 60% suspension in oil) was suspended in dry THF (6 mL) under nitrogen atmosphere and a solution of **TPE-diOH** (364 mg, 1.0 mmol) in dry THF (4 mL) was added in ice-cold condition, and the mixture was stirred for 30 min during which the colorless solution turned yellowish. To this reaction mixture, 1,4-butane sultone (328 mg, 1.2 mmol) was added and stirred vigorously for 10 h at room temperature. The completion of reaction was monitored by TLC. The reaction was quenched with small amount of ice-chips. The crude product mixture was concentrated and evaporated to dryness by keeping in high vacuum to afford crude product. It was first triturated with diethyl ether (2 x 5 mL), the product was taken up in EtOAc (15 mL) and the solid residue was discarded. The product was washed with ice-cold brine solution (2 x 2 mL), dried over anhydrous Na₂SO₄ and concentrated to afford the probe, **TPE-diBuS** as pale yellow solid (410 mg, 60% yield). ¹H NMR (D₂O, 400 MHz), δ (TMS, ppm): 1.35-1.53 (m, 8H), 2.25-2.52 (m, 4H), 2.84-3.01 (m, 4H), 6.15-6.305 (m, 4H), 6.75-7.02 (m, 14H); ¹³C NMR (*d*₆-DMSO, 100 MHz), δ (TMS, ppm): 19.04, 25.50, 48.78, 52.26, 65.43, 69.77, 111.73, 112.37, 126.15, 127.30, 129.05, 130.30, 141.85, 154.15, 154.87; ESI-MS (-ve mode): *m/z* (*Z*= 2) 317.00 (M-Na⁺)/2; HRMS (ESI, -ve mode): calculated for C₃₄H₃₄O₈S₂²⁻ [M/2]²⁻: 317.0853, found 317.0855.

3. Aqueous solubility study of TPE-diBuS

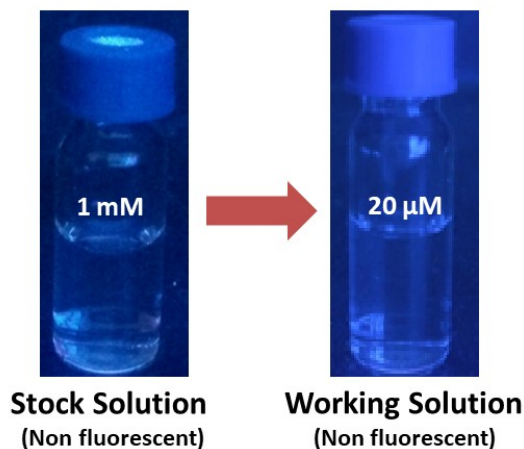


Figure S1. The images of vials taken under short UV are showing that the probe, **TPE-diBuS** has good water solubility and both working solution (20 μM) and stock solution (1 mM) do not show any fluorescence due to AIE-effect making it useful for sensing and imaging studies in organic-solvent-free conditions.

4. pH study of probe (TPE-diBuS) with and without Al³⁺

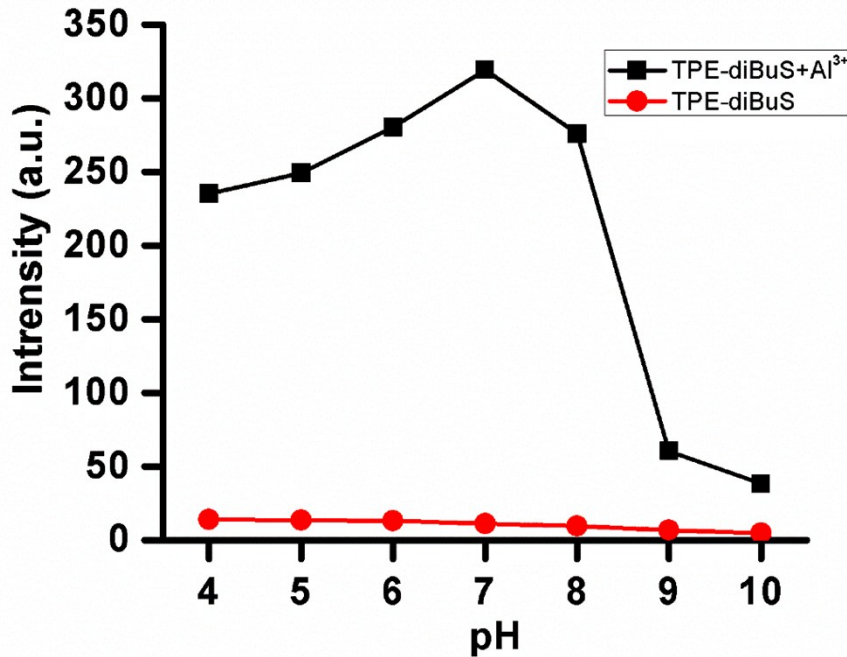


Figure S2. Maximum fluorescence response of **TPE-diBuS** (20 μM) at different pH (pH 4-10) in the presence and absence of Al³⁺ ions (40 μM) [λ_{ex} 345 nm; λ_{em} 468 nm].

5. Association constant calculation

The association constant of the metal complex formed was determined by using the standard Benesi-Hildebrand (B-H).⁴

$$1/(I - I_0) = [1/(I_{\max} - I_0)] [1/\{(Ka)C\} + 1]$$

I_0 is the fluorescence intensity of **TPE-diBuS** at emission maximum ($\lambda_{\text{em}} = 468$ nm), I is the observed fluorescence intensity at that particular wavelength in the presence of a certain concentration of the metal ion (C), I_{\max} is the maximum fluorescence intensity value that was obtained at $\lambda_{\text{em}} = 468$ nm, during titration on adding highest metal ion concentration, K_a is the binding constant and was determined from the slope of the linear plot, and C is the concentration of Al^{3+} ions added during titration experiment.

6. DLS Study

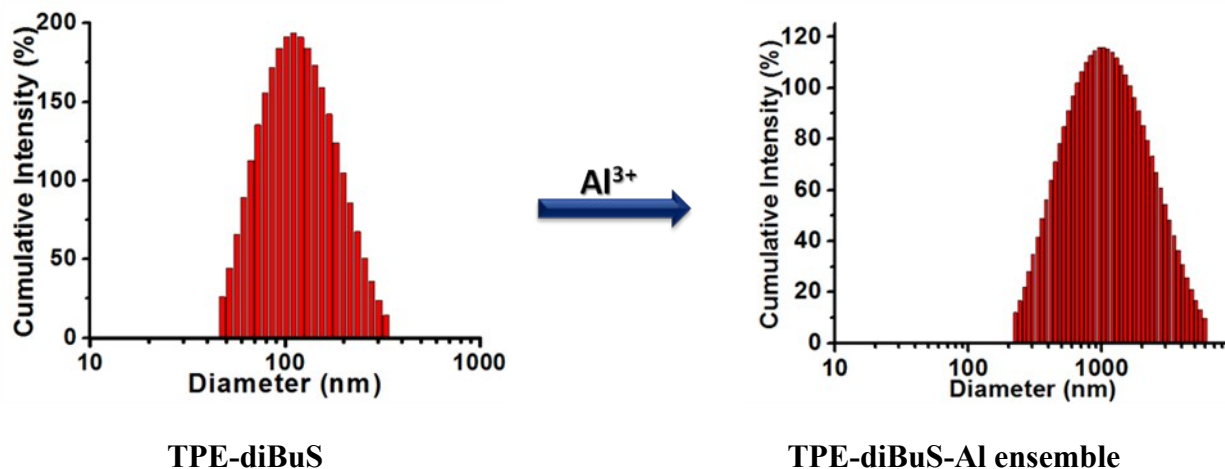


Figure S3. Particle size analysis of **TPE-diBuS** before and after addition of 1.2 equiv of Al^{3+} in water. As expected, the average particle size of **TPE-diBuS** enhance significantly (from 110 nm to 1030 nm) after addition of 1.2 equiv of Al^{3+} to the aqueous solution of **TPE-diBuS** (20 μM) indicating formation of **TPE-diBuS-Al** ensembles.

7. Elemental analysis by EDX

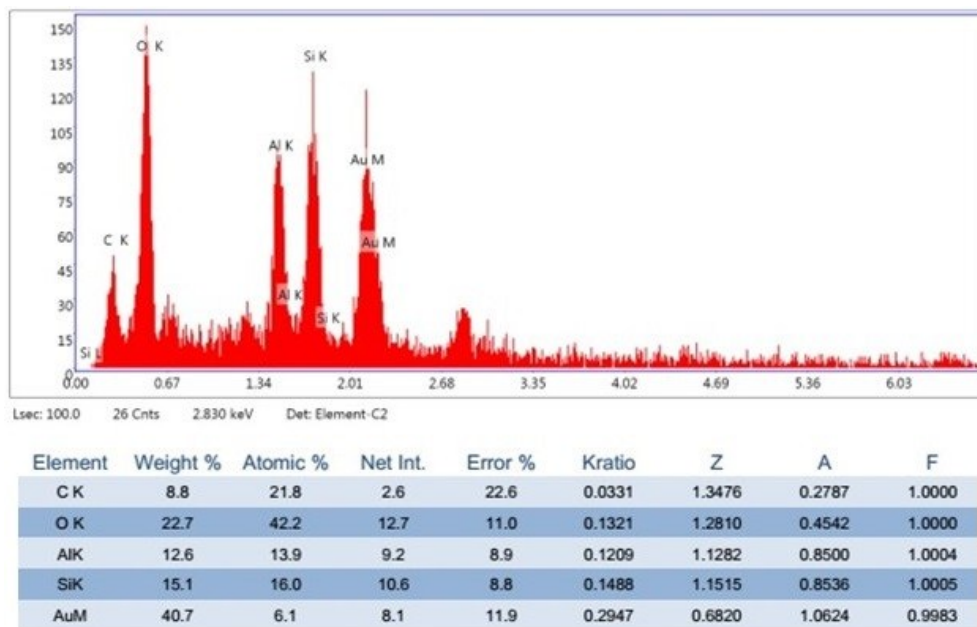


Figure S4. Elemental analysis by EDX was performed to find the real time composition of the system and as mentioned in the chart a good amount of Al, S, O and C were found at different positions of the system.

8. EDTA response towards TPE-diBuS-Al ensemble

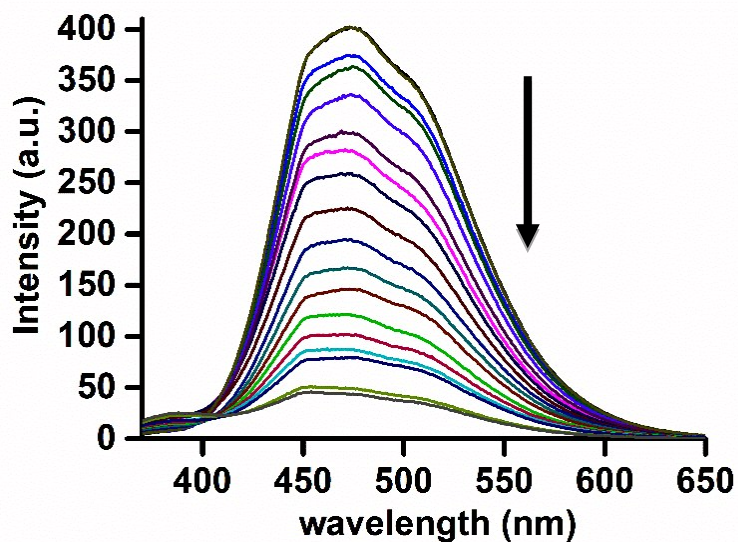


Figure S5. Fluorescence turn-off response of TPE-di-BuS-Al ensemble upon addition of EDTA (0-300 μ M) [λ_{ex} = 345 nm, λ_{em} 468 nm].

9. DFT optimized structures of metal complexes

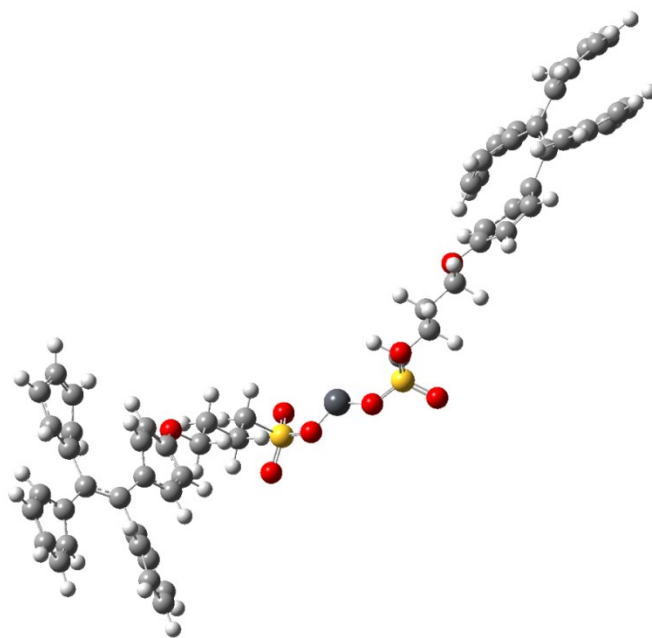


Figure S6. DFT optimized structure of model complex $\text{Pb}(\text{TPE-BuS})_2$.

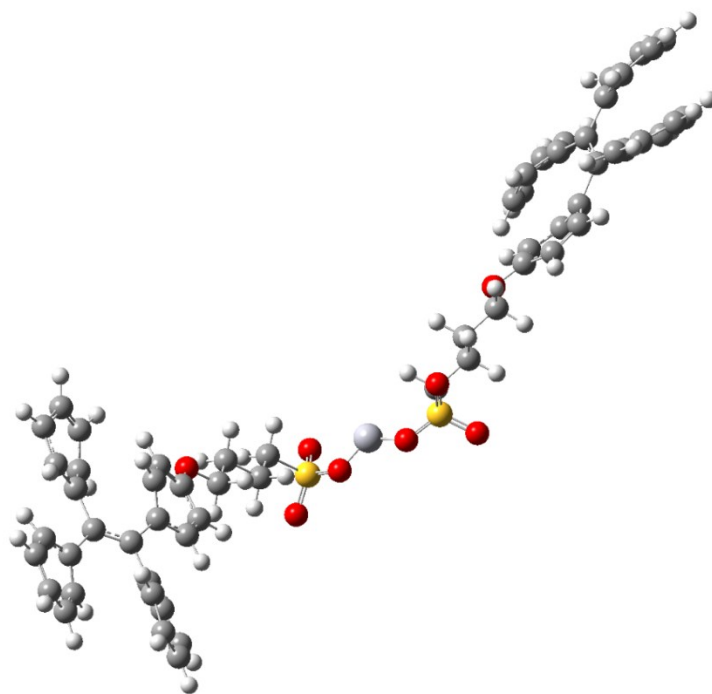


Figure S7. DFT optimized structure of model complex $\text{Hg}(\text{TPE-BuS})_2$.

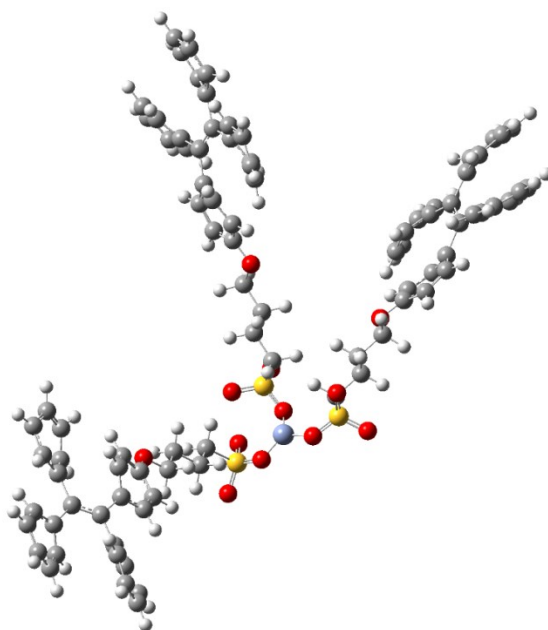


Figure S8. DFT optimized structure of model complex $\text{Cr}(\text{TPE-BuS})_3$.

10. Table S1 Comparison of the present study and previous reports for detection of Al^{3+}

Methods	Solvent System	LOD (μM)	Linear Range (μM)	Ref (Main Manuscript)
Different AIE-gens	THF:Water (25:75 v/v)	1.2	0 - 11	19
	DMSO:Water (2:98 v/v)	0.0216	0.1 - 5	20
	THF-Water (9:1 v/v)	0.33	5.33 – 52.67	21
	MeOH- HEPES pH-7.3, (9:1 v/v)	0.68	--	23(a)
	MeOH- HEPES pH-7.3, (9:1 v/v)	2.8	0 - 50	23(b)
	THF-Water (9:1 v/v)	0.4	0 - 20	24
	MeOH	0.27	0 - 82	25
	WATER	0.05	1 – 20	This Work

11. Limit of quantitation (LOQ) of Al^{3+}

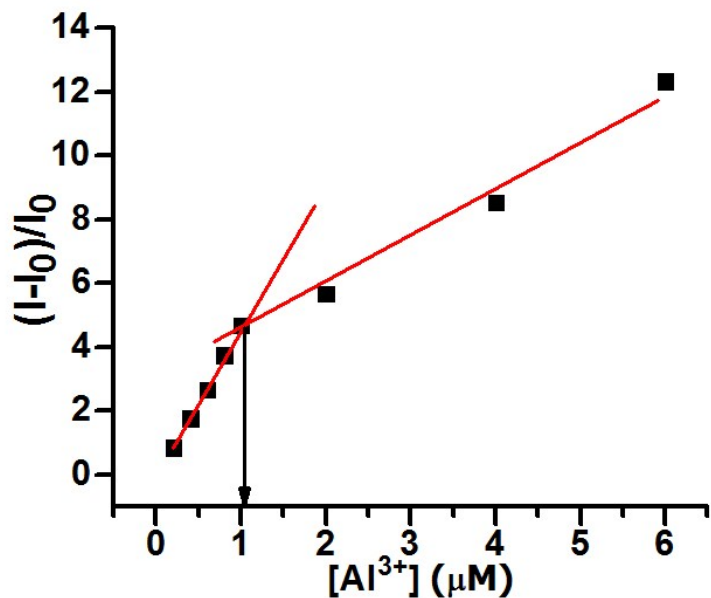


Figure S9. Plot of $[(I-I_0)/I_0]$ v/s increasing concentration of Al^{3+} . LOQ for Al^{3+} was obtained as 1.0 μM .

12. Real sample analysis for Al^{3+} ions

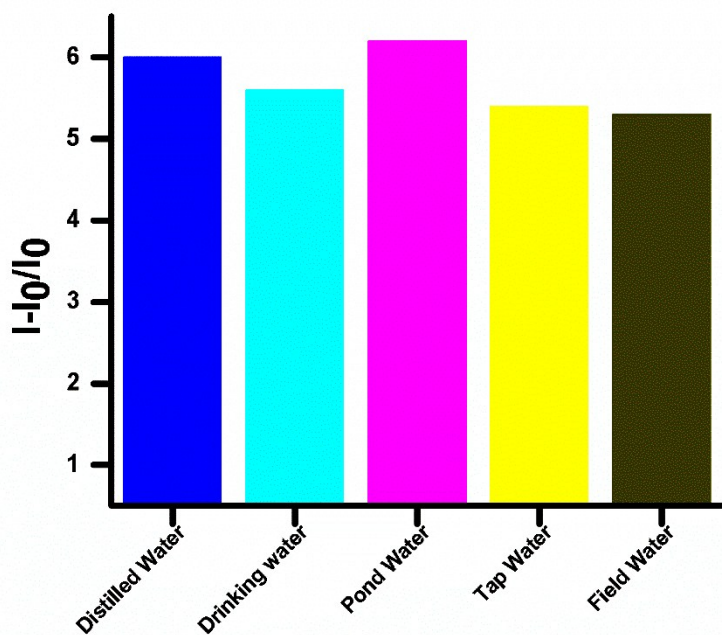


Figure S10. Maximum fluorescence response of **TPE-diBuS** towards the real samples spiked with measured amount of Al^{3+} ions [λ_{ex} 345 nm; λ_{em} 468 nm].

13. Limit of detection (LOD) of F^- ions

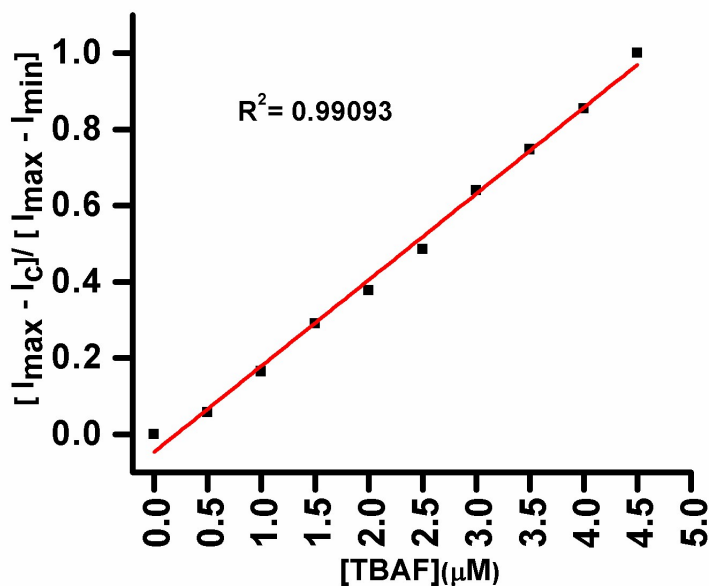


Figure S11. Plot of $[(I_{\text{max}} - I_c) / (I_{\text{max}} - I_{\text{min}})]$ vs increasing concentration of F^- . From the linearity of the plot at lower range of $[\text{F}^-]$ the limit of detection was found as $0.6 \mu\text{M}$.

14. Table S2 Comparison of the present study and previous reports for detection of F^-

Sensors based on displacement ensembles using metal complexes	Solvent System	LOD (μM)	Linear Range (μM)	Main Manuscript
Al^{3+}	MeOH	---	50 - 700	33(a)
Fe^{3+}	30 mM HEPES 30% DMSO (pH 7.2)	140	0 - 500	33(b)
Ca^{2+}	2.5 mM MOPS (pH 7.0)	300	500 - 5000	33(c)
Zr^{4+}	Acetate buffer (pH 5.8)	50	5 - 1000	33(d)
Hg^{2+}	Acetonitrile	0.054	4000-9000	33(e)
Al^{3+}	Hexamethylenetetramine-HCl buffer solution (pH 5)	0.13	---	33(f)
Eu^{3+}	THF	180	10 - 800	33(g)
Al^{3+}	WATER	0.6	0 - 5	This Work

15. Selectivity of anions by TPE-diBuS-Al ensemble

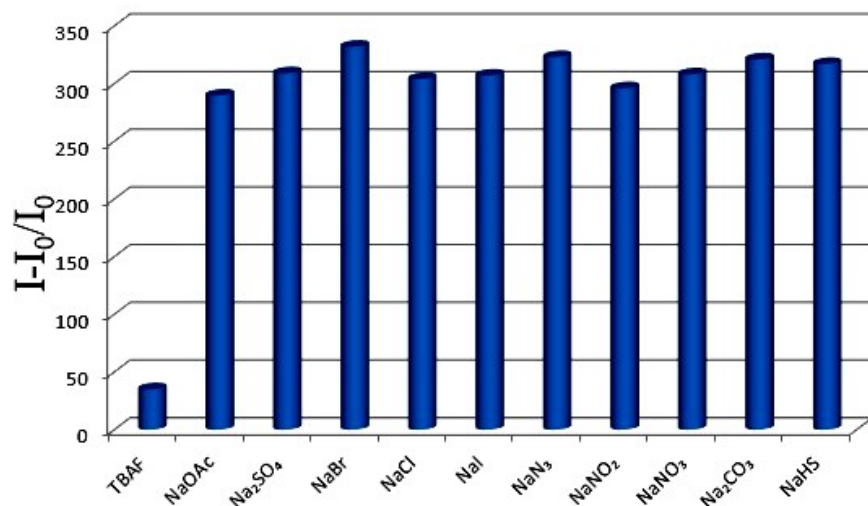


Figure S12. Effect of various anions on the fluorescence intensity of **TPE-diBuS-Al** ensemble. As shown, the ensemble can selectively respond to F⁻ in the presence of many other competing anions.

16. Cytotoxicity data of TPE-diBuS

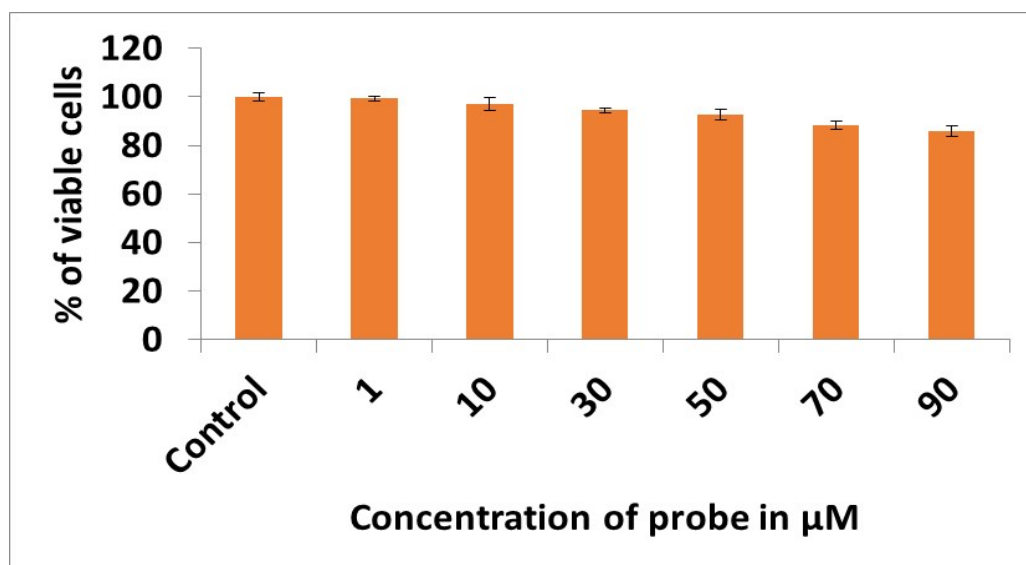


Figure S13. Cytotoxicity by MTT assay of **TPE-diBuS** at different concentrations is presented above. The bar graphs showed that more than 90% of the total cells were still viable in the presence of up to 90 μM of **TPE-diBuS** in the culture media of HK cells after 24 h. This implies that the **TPE-diBuS** is nontoxic in nature.

16. Wash-free imaging

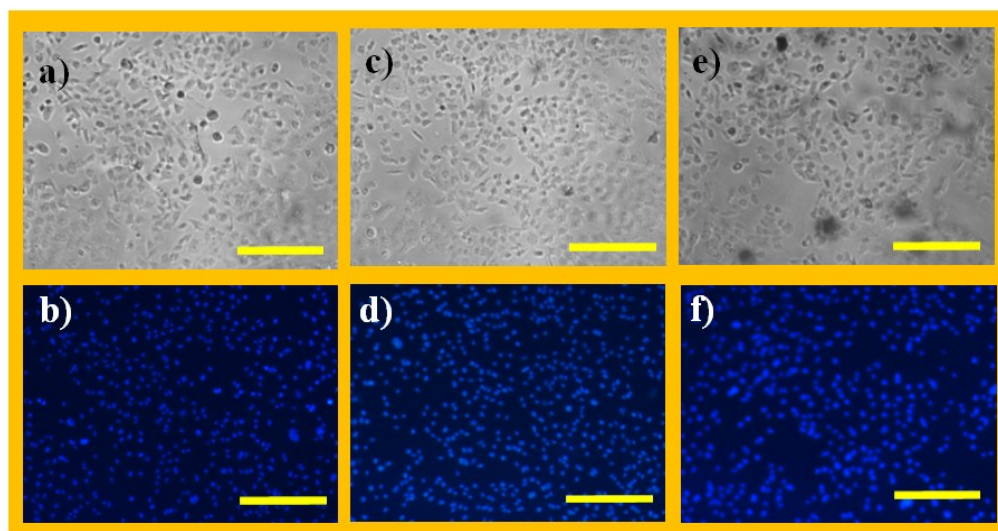
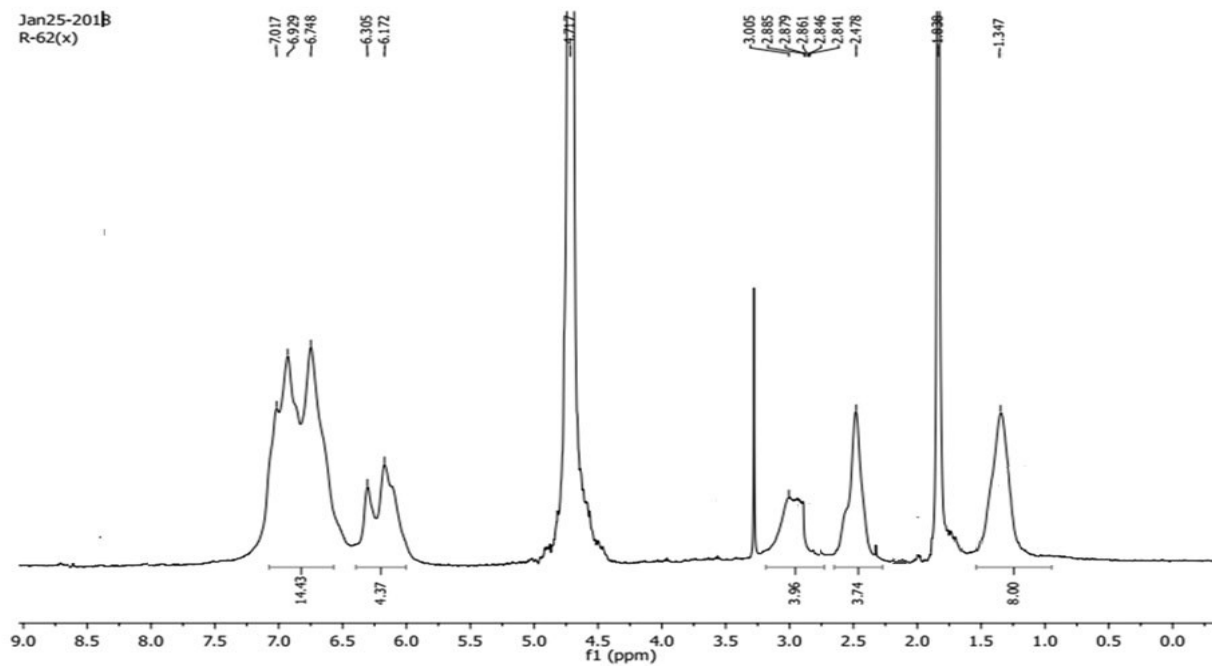


Figure S14. Bright field and fluorescence images of HK cells pretreated with different concentration of Al^{3+} (a,b) 1 μM , (c,d) 5 μM , (e,f) 10 μM and upon subsequent treatment with 10 μM of **TPE-diBuS** and incubation for 1 h. The images were captured using a digital camera (Canon) with 16x resolution. The scale bar is 1 μm .

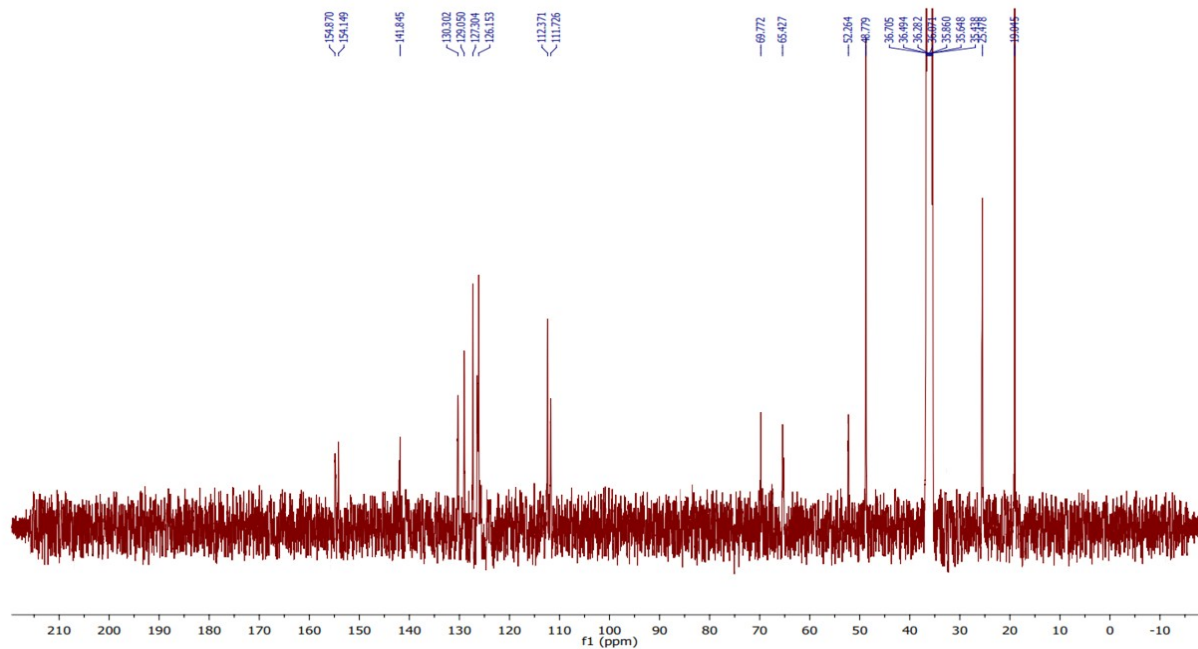
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¹H NMR of TPE-diBuS



¹³C NMR of TPE-diBuS



HRMS spectrum for TPE-diBuS

