

SUPPLEMENTARY INFORMATION

A simple Schiff base platform: Sensing of Al³⁺ ions in an aqueous medium

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Experimental Procedure

Cell culture

NCCS cells were cultured RPMI-1640 supplemented with 10% fetal bovine serum (FBS) at 37 °C under an atmosphere of 5% CO₂. Cells were plated on 18 mm glass cover slips and allowed to adhere for 24 h.

Cytotoxicity assay

The methyl thiazolyl tetrazolium (MTT) assay was used to measure the cytotoxicity of probe towards NCCS cells. The cells were seeded into a 96-well cell-culture plate. Various concentrations (10, 20, 30, 40 and 50 μM) of PADP-probe were added to the wells. The cells were incubated at 37°C under 5% CO₂ for 24 h. 10 μL MTT (5 mg/mL) was added to each well and incubated at 37°C under 5% CO₂ for 4 h. Multiskan GO microplate reader was used to measure the absorbance at 510 nm for each well. The viability of cells was calculated according to the following equation: Cell viability (%) = (mean of absorbance value of treatment group) / (mean of absorbance value of control group).

Cell imaging

NCCS cells were initially cultured in a 25 cm² tissue culture flask containing RPMI-1640 medium supplemented with 10% FBS, penicillin (100 μg mL⁻¹) and streptomycin (100 μg mL⁻¹) in a CO₂ incubator. Prior to imaging studies, the cells were seeded into a 6 well plate and grown in RPMI-1640 medium at 37°C till 80% confluency in CO₂ incubator. Subsequently, the cells were washed thrice with sterile phosphate buffered saline (PBS), incubated with 10 μM of PADP-probe in RPMI-1640 at 37°C for 1 h in a CO₂ incubator. The cells were again washed with sterile PBS to remove excess probe and their images were acquired using a fluorescence microscope (Eclipse Ti-U, Nikon, USA) with a filter that allowed green light emission. The cells were subsequently incubated with sterile PBS separate set with 20 μM AlNO₃ for 1 h. The images of the cells were acquired with a fluorescence microscope.

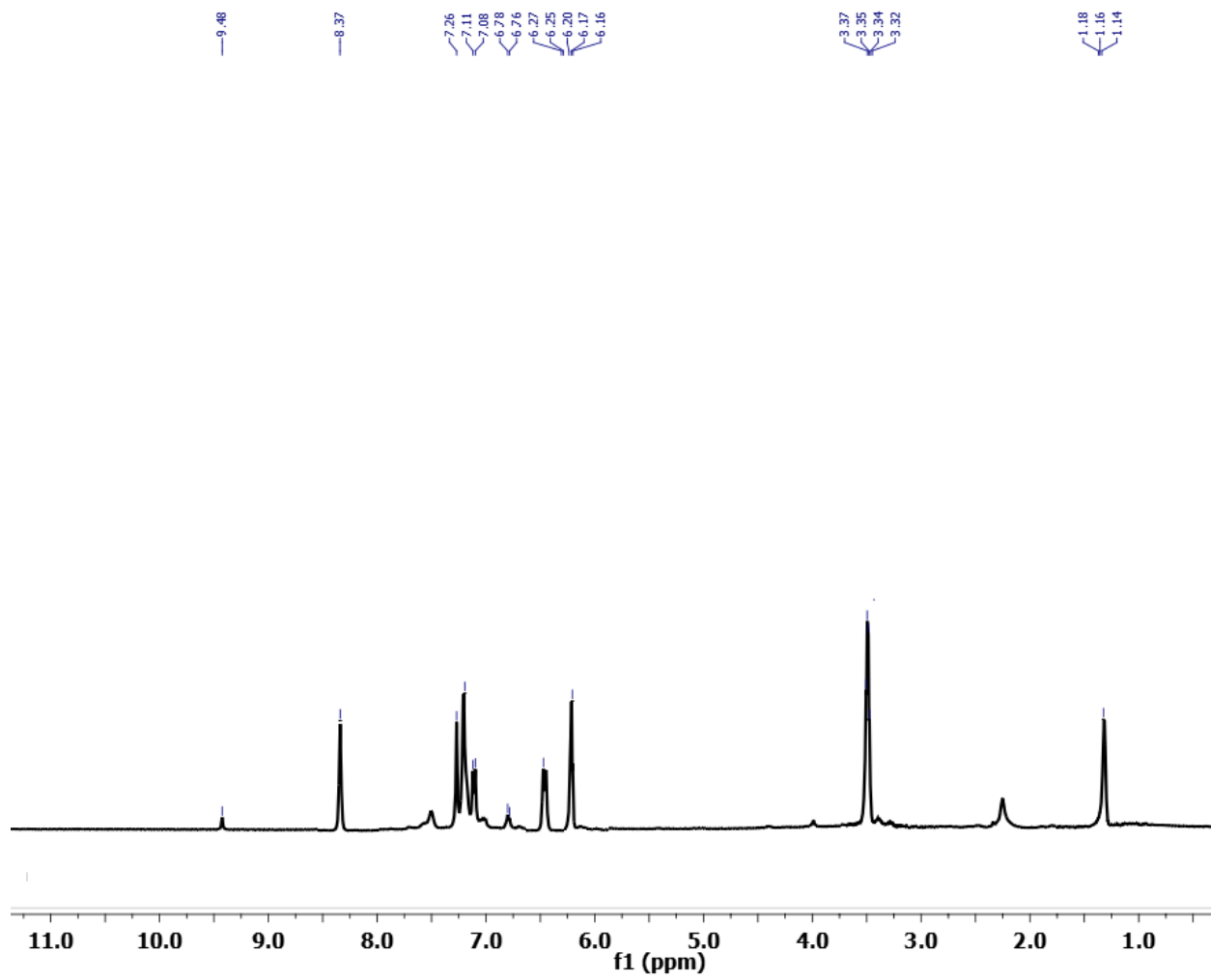


Figure S1. ^1H NMR spectrum of PADP

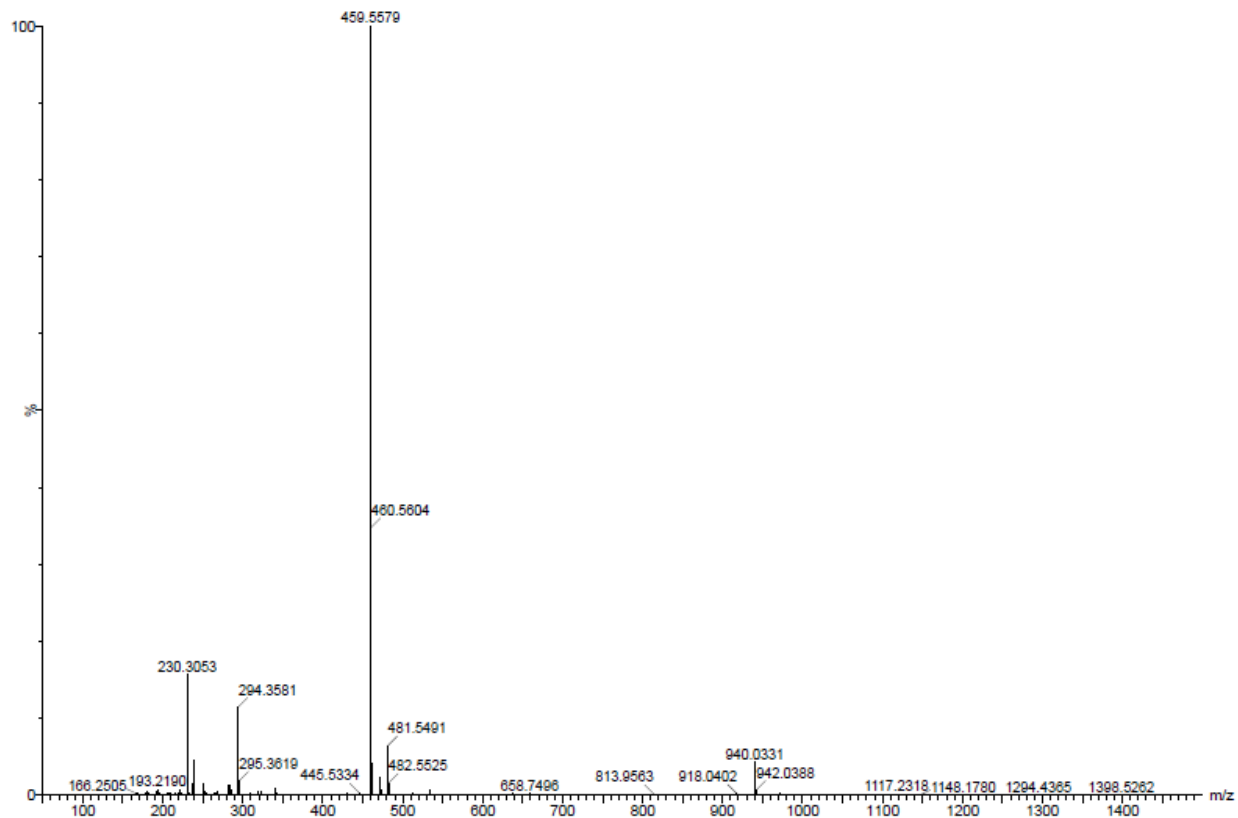


Figure S2. Mass spectrum of PADP

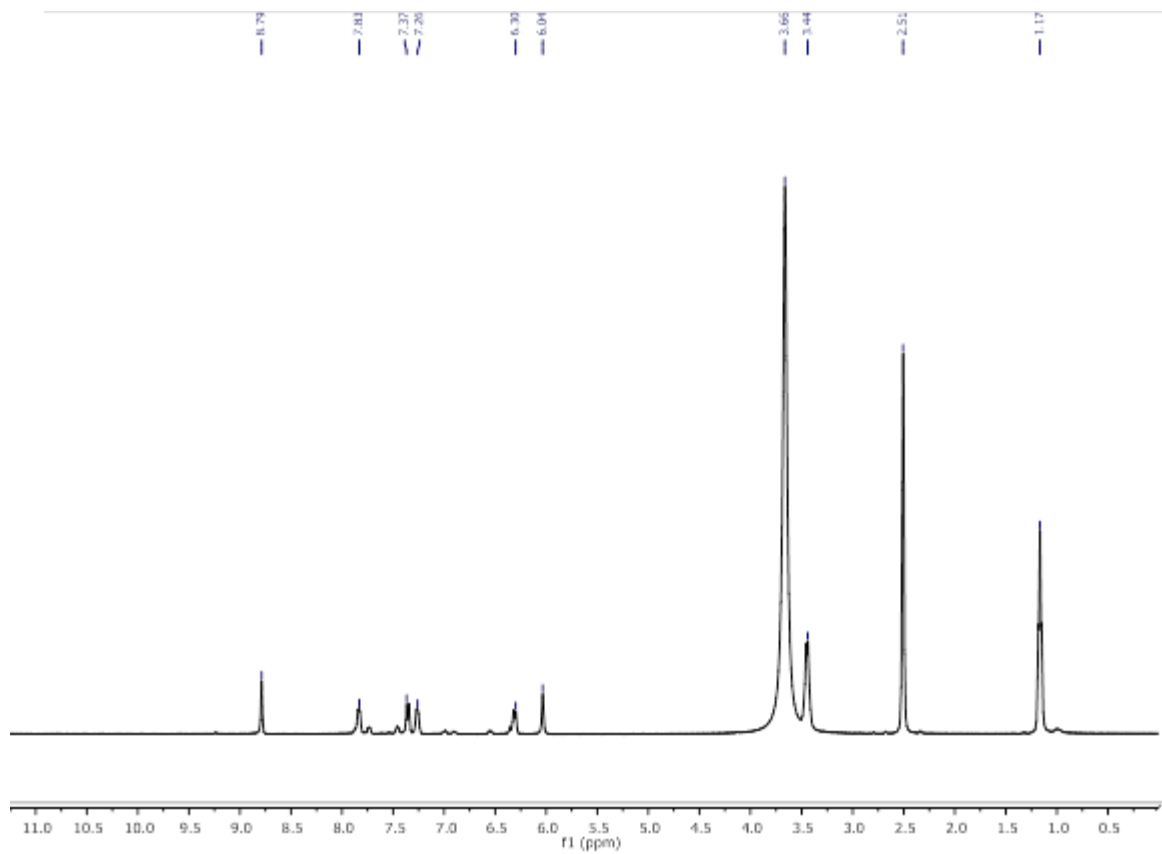


Figure S3. ¹H NMR spectrum of PADP+ Al³⁺

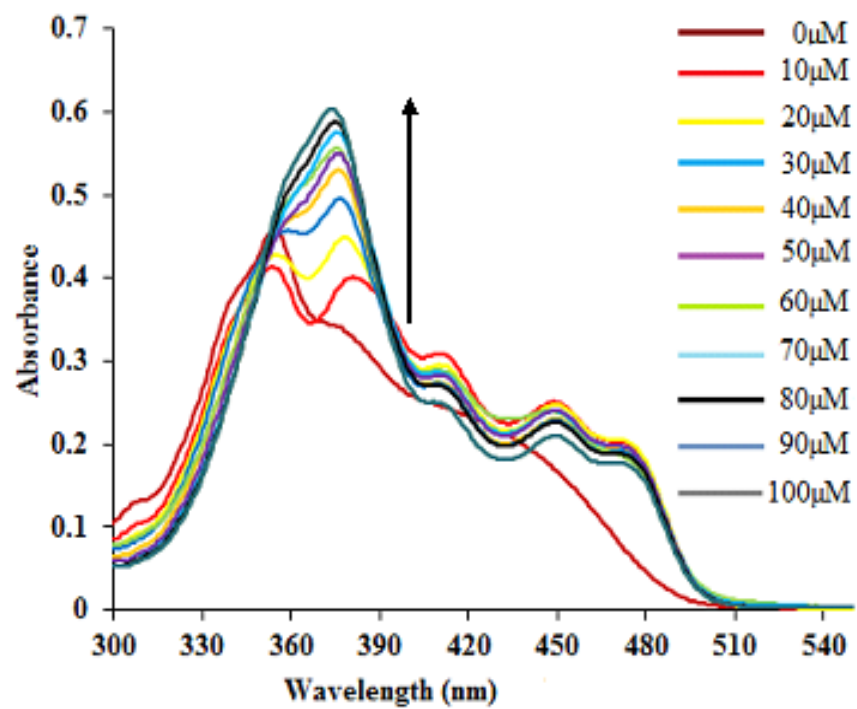


Figure S4. UV-Vis spectrum of the probe PADP (10 μM) in MeOH/H₂O (1:9 (v/v), HEPES=50mM, pH=7.4) with 0-100 μM of Al³⁺.

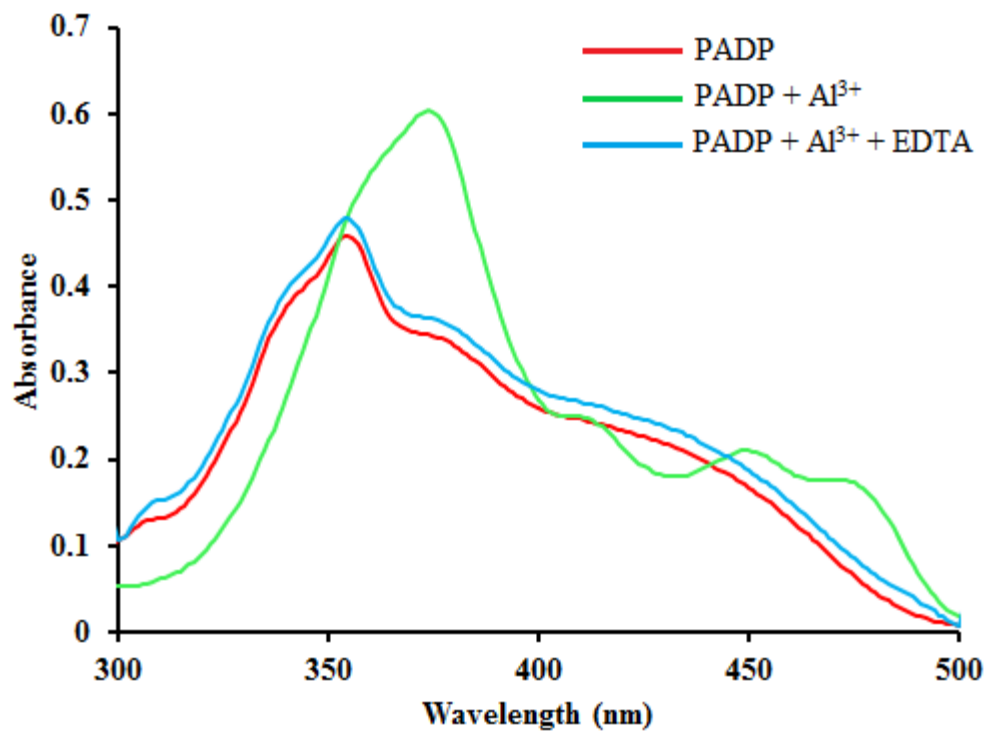


Figure S5. UV-Vis spectrum of the probe PADP (10 μM) in MeOH/H₂O (1:9 (v/v), HEPES=50mM, pH=7.4), PADP+Al³⁺ and PADP+Al³⁺+EDTA.

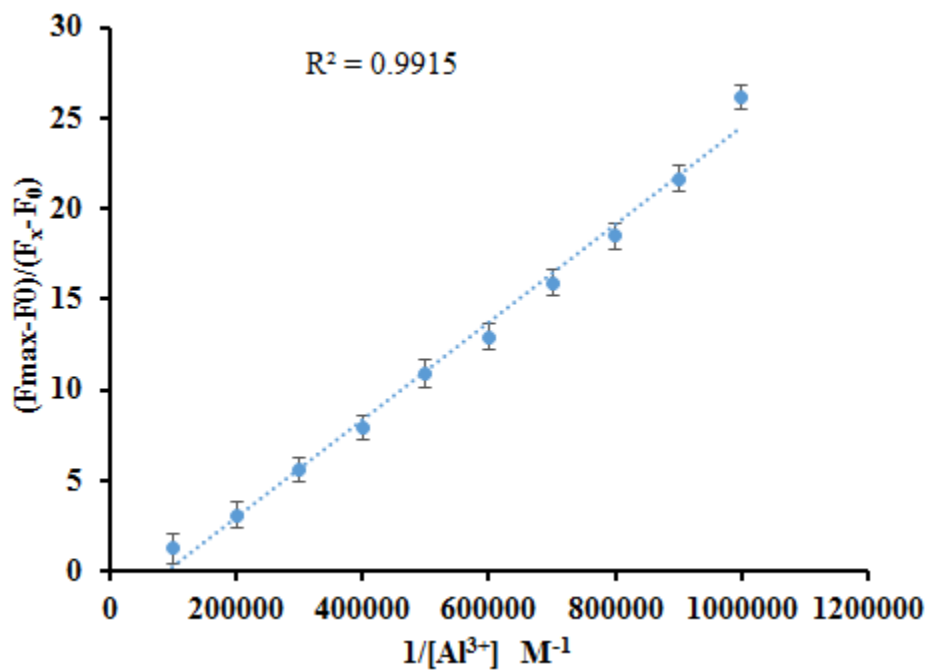


Figure S6. Benesi-Hilderbrand plot of PADP in MeOH/H₂O (1:9 (v/v), HEPES=50mM, pH=7.4) ($\lambda_{\text{ex}}= 450 \text{ nm}$)

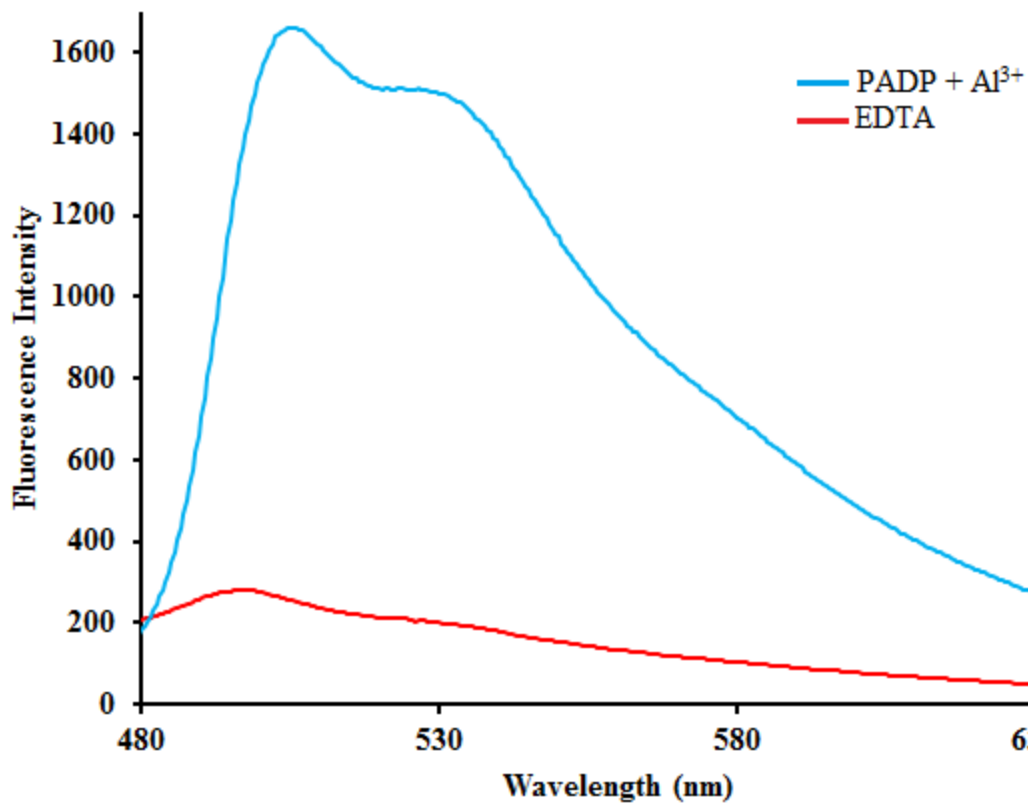


Figure S7. Fluorescence response of the PADP-Al³⁺ complex (10 μ M) in MeOH /H₂O (1:9 (v/v), 50 mM HEPES, pH=7.4) with 100 μ M of EDTA (λ_{ex} =450 nm).

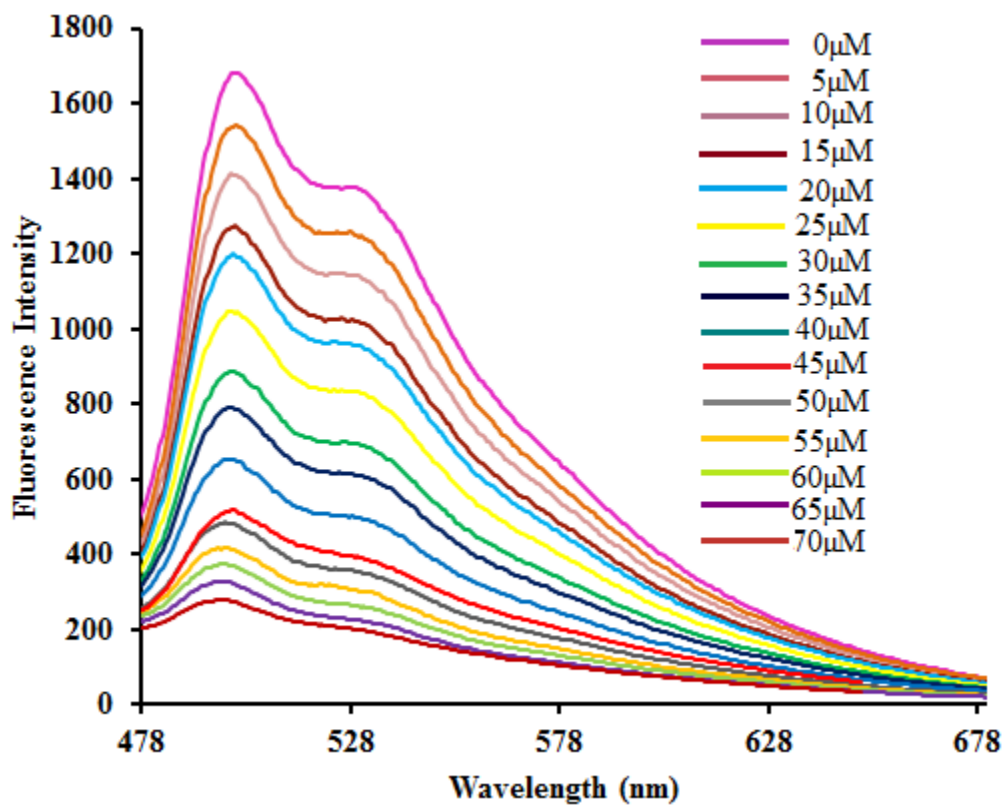


Figure S8. Fluorescence responses of the PADP+Al³⁺ (10 μM) in MeOH/H₂O (1:9 (v/v), HEPES=50mM, pH=7.4) with 0-70 μM of EDTA (λ_{ex} =450 nm).

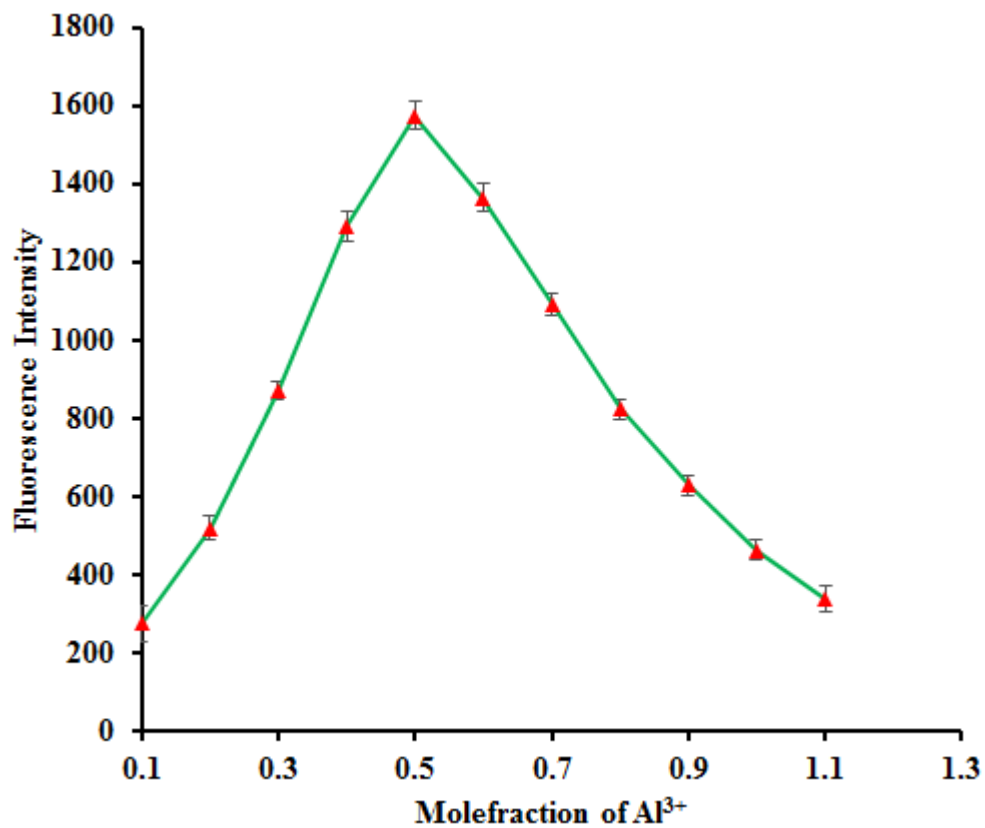


Figure S9. Job's plot for the determination between PADP and Al³⁺ in MeOH/H₂O (1:9 (v/v), HEPES=50mM, pH=7.4) (λ_{ex} = 450 nm)

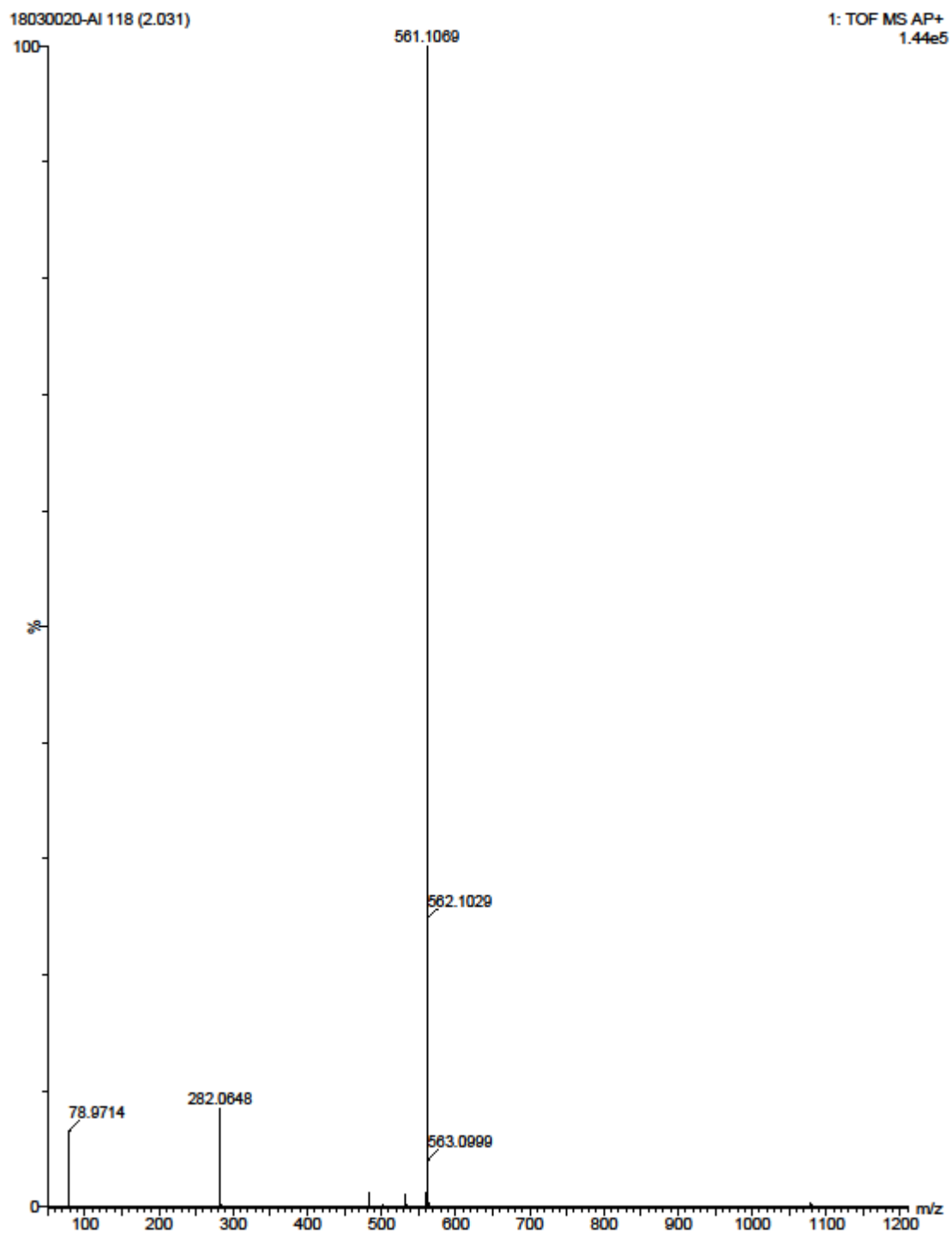


Figure S10. Mass spectrum of PADP - Al³⁺ complex

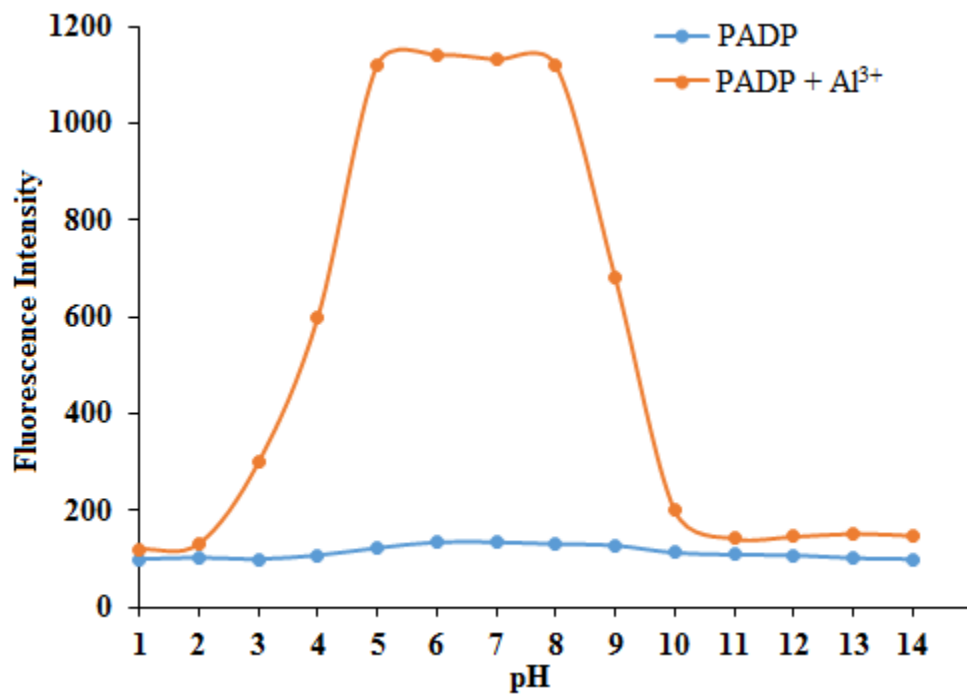


Figure S11. Fluorescence response of the PADP and PADP- Al³⁺ complex in MeOH/H₂O (1:9 (v/v) in various range of pH (1-14) (λ_{ex} =450 nm).

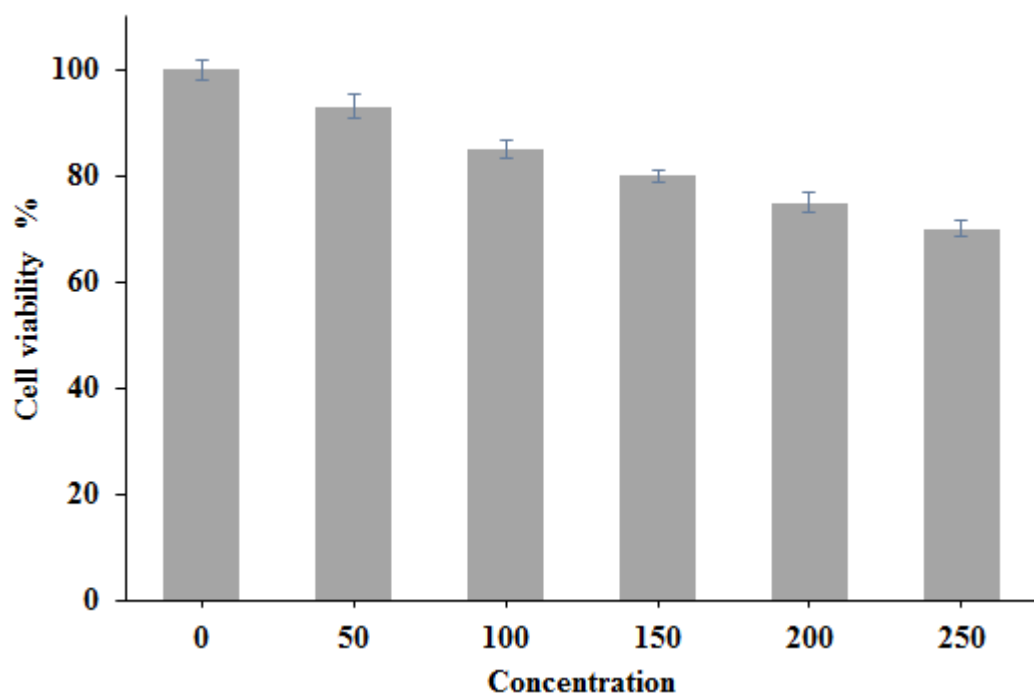


Figure S12. Cytotoxicity assay for the probe PADP in different concentration medium (0, 50, 110, 150, 200, 250 μ M) in NCCS cells.

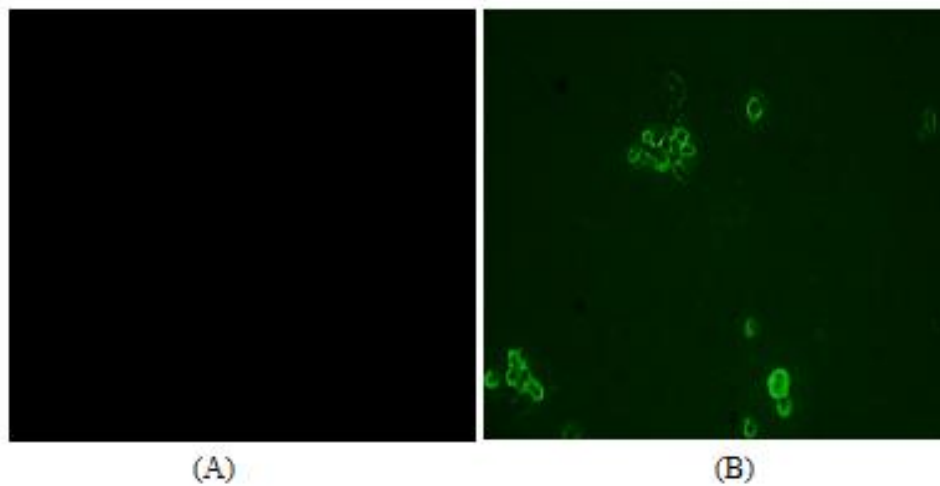


Figure S13. Fluorescence images of NCCS cells treated with (A) PADP (10 μ M) (B) PADP and Al³⁺ ions (15 μ M).

Table S1. The comparison of **PADP** with some other probes for Al³⁺ ions.

Mechanism	Solvent(v/v)	LOD(μ M)	Application	References
FRET	aqueous	0.5	-	[1]
ESIPT	DMSO	2.40	cell imaging	[2]
CHEF	CH ₃ OH-H ₂ O	0.60	-	[3]
ESIPT	methonal	5.98	cell imaging	[4]
PET	ethonal-water(3:1)	0.182	-	[5]
PET,ESIPT	aqueous	4.0	-	[6]
ICT,CHEF	methonal	6.9	-	[7]
PET,CHEF	methonal-water(1:9)	0.104	Real sample analysis and live cell imaging	present work

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Table S2: Determination of Al³⁺ ions in real water samples.

Sample	Al³⁺ spiked (μM)	Al³⁺recovered (μM)	Recovery (%)
River water 1	2	1.97	98.5
River water 2	4	3.92	98
River water 3	6	5.95	99.1
Tap water 1	2	1.96	98
Tap water 2	4	3.93	98.25
Tap water 3	6	5.92	98.67