Supporting Information

for

A rhodamine-based 'turn-on' Al³⁺ ion-selective reporter and the resultant complex as a secondary sensor for F⁻ ion are applicable to living cell staining[†]

Buddhadeb Sen,^a Manjira Mukherjee,^a Samya Banerjee,^b Siddhartha Pal^a and Pabitra Chattopadhyay*^a

^aDepartment of Chemistry, Burdwan University, Golapbag, Burdwan, 713104, India

^bDepartment of Inorganic and Physical Chemistry, Indian Institute of Science, Bangalore, 560012, India

Corresponding author: <u>pabitracc@yahoo.com</u>

CONTENTS

IR, ESI-MS, ¹ H and ¹³ C NMR spectrum of L	Figs. S1-S4
IR, ESI-MS, ¹ H and ¹³ C NMR spectrum of L'-AI complex	Figs. S5-S8
pH study	Fig. S9
Selectivity study	Fig. S10
Fluorescence color change of the probe in absence and presence of different metal ions	Fig. S11
Interference of different ions in presence of L and Al ³⁺ ions	Fig. S12
Job's plot from the emission data	Fig. S13
Binding constant (K) plots	Fig. S14
LOD graph	Fig. S15
¹ H NMR titration in DMSO-d ₆	Fig. S16
Some HOMO-LUMO's of L and L'-Al complex	Fig. S17-S18
¹ H NMR spectrum of 2 in presence of F ⁻ ions in DMSO-d ₆	Fig. S19
Cell imaging study	Figs. S20, S22, S24
Cell Cytotoxicity Assay	Figs. S21 & S23
Crystallographic data table	Table S1-S2
Life time details	Table S3
Energies and Key transitions from TDDFT calculations	Table S4-S6

Materials and physical methods

General Procedures

High-purity HEPES, o-nitrobenzaldehyde and Al(NO₃)₃.6H₂O were purchased from Sigma Aldrich (India) and rhodamine B from E. Merck, solvents used were spectroscopic grade. All metal salts were used as their nitrate, acetate, sulphate or their chloride salts. Other chemicals were of analytical reagent grade and used without further purification except when specified. Milli-Q, 18.2 M Ω cm⁻¹ water was used throughout all experiments. A Shimadzu (model UV-1800) spectrophotometer was used for recording electronic spectra. FTIR spectra were recorded using Perkin Elmer FTIR model RX1 spectrometer preparing KBr disk. ¹HNMR spectrum of organic moiety was obtained on a Bruker Avance DPX 500 MHz spectrometer using DMSO-d₆ solution. Electrospray ionization (ESI) mass spectra were recorded on a Qtof Micro YA263 mass spectrometer. A Systronics digital pH meter (model 335) was used to measure the pH of the solution and the adjustment of pH was done using either 50 mM HCl or NaOH solution. Steadystate fluorescence emission and excitation spectra were recorded with a Hitachi-4500 spectrofluorimeter. Time-resolved fluorescence lifetime measurements were performed using a HORIBA JOBIN Yvon picosecond pulsed diode laser-based time-correlated single-photon counting (TCSPC) spectrometer from IBH (UK) at λ_{ex} = 550 nm, and MCP-PMT as a detector. Emission from the sample was collected at a right angle to the direction of the excitation beam maintaining magic angle polarization (54.71). The full width at half-maximum (FWHM) of the instrument response function was 250 ps, and the resolution was 28.6 ps per channel. Data were fitted to multiexponential functions after deconvolution of the instrument response function by an iterative reconvolution technique using IBH DAS 6.2 data analysis software in which reduced w2 and weighted residuals serve as parameters for goodness of fit.

Emission study

Quantum Yield Calculation

Organic moiety (L) shows a very weak emission at 590 nm in HEPES buffer (1 mM, ethanol/water: 1/9, v/v; pH 7.4) at 25 °C when excited at 525 nm considering the absorption hump at 525 nm. Fluorescence quantum yields (Φ) were estimated by integrating the area under the fluorescence curves with the equation:

$$\Phi_{\text{sample}} = \Phi_{\text{ref}} X \quad \frac{\text{OD}_{\text{ref}} x \text{ A}_{\text{sample}} x \Pi^2_{\text{sample}}}{\text{OD}_{\text{sample}} x \text{ A}_{\text{ref}} x \Pi^2_{\text{ref}}}$$

where A is the area under the fluorescence spectral curve and OD is optical density of the compound at the excitation wavelength, 525 nm, Π is the refractive index of the solvent used. The standard used for the measurement of fluorescence quantum yield was rhodamine-B ($\Phi = 0.7$ in ethanol).

In case of L the fluorescence quantum yield has been calculated in absence and presence of Al^{3+} ions and from this measurement it is clear that the fluorescence quantum yield increases more than ~25 times (at λ = 590 nm) upon addition of 2.0 equivalent of Al^{3+} ions.

The luminescence property of the sensor was investigated in water-EtOH (3 : 1, v/v) solvent. The pH study was done in 1 mM HEPES buffer solution by adjusting pH with HCl or NaOH. The stock solutions (10^{-2} M) for the selectivity study towards different ions were prepared in water-EtOH (3 : 1, v/v) solvent. In the selectivity studies the concentration of the ions was a hundred times greater than that of the probe used. Fluorescence titration was performed with aluminium nitrate in water-EtOH (3:1, v/v) solvent, varying the metal concentration 0 to 20 µM; probe concentration was 10 µM. The in *vivo* studies were performed at biological pH-7.4 in PBS buffer solution.



Fig. S1 FT-IR spectrum of L



Fig. S2 ESI-MS spectrum of L



Fig. S3 ¹H NMR spectrum of L in DMSO-d₆



Fig. S4 ¹³C NMR spectrum of L in DMSO-d₆



Fig. S5 FT-IR spectrum of L'-Al complex (2)



Fig. S6 ESI-MS spectrum of L'-Al complex (2)



Fig. S7 ¹H NMR spectrum of L'-Al complex in DMSO-d₆



Fig. S8 ¹³CNMR spectrum of **L'-Al** complex in DMSO-d₆ [The peak at 66.424 ppm due to spiro lactum carbon (C₃, *viz*. Fig. S4) has been vanished]



Fig. S9 Fluorescence response of L (10 μ M) in absence and in presence of Al³⁺ ions (10 μ M) in aqueous EtOH: water (1:3) media at different pH at $\lambda_{em} = 590$ nm.



Fig. S10 Fluorescence intensity assay of L (10 μ M) in presence of different metal ion salts in HEPES buffer (1 mM, pH 7.4) at 25 °C ($\lambda_{ex} = 525$ nm), a) Na⁺, b) K⁺, c) Ca²⁺, d) Mg²⁺, e) Cr³⁺, f) Mn²⁺, g) Fe³⁺, h) Co²⁺, i) Ni²⁺, j) Cu²⁺, k) Zn²⁺, l) Cd²⁺, m) Hg²⁺, n) Al³⁺, and o) Pb²⁺ at $\lambda_{em} = 590$ nm.



Fig. S11 Fluorescence color change of the probe in absence and presence of different metal ions in EtOH : water (1:3) HEPES buffer (1 mM, pH 7.4) at 25°C in presence of UV-light.



Fig. S12 Interference of different ions (100 μ M) in presence of L (10.0 μ M) and Al³⁺ ions (10.0 μ M) in EtOH : water (1:3) HEPES buffer at 25 °C ($\lambda_{ex} = 525$ nm), a) Na⁺, b) K⁺, c) Ca²⁺, d) Mg²⁺, e) Cr³⁺, f) Mn²⁺, g) Fe³⁺, h) Co²⁺, i) Ni²⁺, j) Cu²⁺, k) Zn²⁺, l) Cd²⁺, m) Hg²⁺ and n) Pb²⁺ at $\lambda_{em} = 590$ nm.



Fig. S13 Job's plot from the emission data for stoichiometry determination between L and Al³⁺ ions in EtOH: water (1:3) at 25 °C at $\lambda_{em} = 590$ nm.



Fig. S14 Binding constant (K) value 8.13 x 10⁴ M⁻¹ determined from the intercept/slope of the plots resulting in the interactions of L with Al³⁺ ions at $\lambda_{em} = 590$ nm.



Fig. S15 Calibration graph in the nano molar linearity region with error bar for the detection of Al^{3+} ions in EtOH : water (1:3) at 25 °C at $\lambda_{em} = 590$ nm.



Fig. S16 Partial ¹H NMR titration of L in presence of varying [Al³⁺] [A) 0, B) 0.5, and C) 1 equivalent] in DMSO-d₆



Fig. S17 Some HOMO and LUMO's of L (H atoms are omitted for clarity).



Fig. S18 Some HOMO and LUMO's of L'-Al complex (H atoms are omitted for clarity).



Fig. S19 ¹H NMR spectrum of L'-Al complex in presence of F⁻ ions (1 equivalent) in DMSO-d₆



Fig. S20 Fluorescence and phase contrast image of HeLa cells after incubation with L (10 μ M) in presence of Al³⁺ ions 1) 0 μ M, 2) 5 μ M, 3) 7 μ M, 4) 10 μ M for 30 min at 37°C ($\lambda_{ex} = 530$ nm).



Fig. S21 Cytotoxic effect of **L** in HeLa cells incubated for 6 h by MTT assay. Results are expressed as mean of three independent experiments.



Fig. S22 Fluorescence and phase contrast image of breast cancer cells (MCF-7) after incubation with L (10 μ M) in presence of Al³⁺ ions 1) 0 μ M, 2) 5 μ M, 3) 10 μ M for 30 min at 37°C (λ_{ex} = 530 nm).



Fig. S23 Cytotoxic effect of L in MCF-7 cells incubated for 6 h by MTT assay. Results are expressed as mean of three independent experiments.



Fig. 24 Fluorescence image and phase contrast image of HeLa cells after incubation with 10 μ M of L'-Al complex followed by NaF (1) 0 μ M, (2) 5 μ M, (3) 10 μ M and (4) 50 μ M respectively for 30 min at 37°C ($\lambda_{ex} = 530$ nm).

	L
Empirical Formula	C ₃₅ H ₃₅ N ₄ O ₃
Formula Weight	589.68
Crystal System	Triclinic
Space group	P-1
a (Å)	9.738(2)
b (Å)	11.472(3)
c (Å)	15.671(4)
α (°)	80.149(4)
β (°)	80.410(4)
γ (°)	65.723(3)
Density (mg/m ³)	1.253
Volume (Å ³)	1563.3(7)
Temperature, K	296(2)
Ζ	2
F (000)	624
θ range(deg)	1.326 to 25.027
Collected reflections	22055
Independent reflections	5534
Goodness-of-fit	1.001
R1 [I > 2.0 σ (I)]	0.0725
wR1 [I > 2.0 σ (I)]	0.1920

Table S1 Crystal data and details of refinements for L

Bond distances (Å)						
1.382(3)	C28-C29	1.492(6)				
1.366(3)	C32-C33	1.408(7)				
1.497(3)	C8-C9	1.473(4)				
1.202(3)	C23-C24	1.402(4)				
1.390(4)	C15-C16	1.522(3)				
1.482(6)	C19-C20	1.410(4)				
Bond an	ngles (°)					
111.31(19)	C14-C15- C27	111.49(19)				
118.2(2)	C29-N4-C30	117.0(3)				
127.94(19)	O4-C22- C23	114.8(2)				
118.0(2)	N4-C19- C20	121.3(3)				
126.0(3)	O3-C8-N3	125.6(3)				
121.4(2)	C27-C22- C23	123.3(2)				
121.3(3)	C24-C25- C26	121.0(2)				
	Bond dist 1.382(3) 1.366(3) 1.497(3) 1.202(3) 1.390(4) 1.482(6) Bond an 111.31(19) 118.2(2) 127.94(19) 118.0(2) 126.0(3) 121.4(2) 121.3(3)	Bond distances (Å) $1.382(3)$ C28-C29 $1.366(3)$ C32-C33 $1.497(3)$ C8-C9 $1.202(3)$ C23-C24 $1.390(4)$ C15-C16 $1.482(6)$ C19-C20Bond angles (°) $111.31(19)$ C14-C15- C27 $118.2(2)$ C29-N4-C30 $127.94(19)$ O4-C22- C23 $118.0(2)$ N4-C19- C20 $126.0(3)$ O3-C8-N3 $121.4(2)$ C24-C25- C26				

Table S2 Selected bond distances (Å) and bond angles (°) for L

Table S3 Life time detail of L (10 μ M) at 590 nm in absence and presence of Al³⁺ ions

	B1	B2	$\tau_1(ns)$	$\tau_2(ns)$	τ _{en} (ns)	v ²	0	Kr	Knr	Kr / Knr
L	95.29	4.71	0.096	1. 33	0.154	x 1.00	0.02	0.129	6.36	0.02
$L + Al^{3+}$ ions (1:0.5)	59.36	40.64	0.799	1.22	0.971	1.04	-	-	-	-
$L + Al^{3+}$ ions(1:1)	-	100	1.04	-	1.04	1.07	0.51	0.490	0.471	1.04

]	L	L'-Al Complex		
MO's	Energy (eV)	MO's	Energy (eV)	
LUMO+5	-0.27	LUMO+5	-1.31	
LUMO+4	-0.42	LUMO+4	-2.47	
LUMO+3	-0.56	LUMO+3	-2.62	
LUMO+2	-1.06	LUMO+2	-2.78	
LUMO+1	-1.91	LUMO+1	-2.90	
LUMO	-2.70	LUMO	-3.57	
НОМО	-5.24	НОМО	-5.68	
HOMO-1	-5.29	HOMO-1	-6.47	
HOMO-2	-6.06	HOMO-2	-6.78	
HOMO-3	-6.39	HOMO-3	-7.06	
HOMO-4	-7.08	HOMO-4	-7.45	
HOMO-5	-7.34	HOMO-5	-7.67	

 Table S4 The energy values of some HOMO-LUMO's of L and L'-Al Complex

E _{excitation} (eV)	$\lambda_{\text{excitation}}$ (nm)	Osc. strength (f)	Key transitions	CI	λ _{expt.} (nm)
1.5607	794.41	0.0030	$HOMO \rightarrow LUMO$	0.61110	
2.3254	533.16	0.0010	HOMO-2 \rightarrow LUMO	0.70547	
2.6878	461.28	0.1813	HOMO-3 \rightarrow LUMO	0.69744	
2.8393	436.68	0.0090	HOMO \rightarrow LUMO+1	0.57062	
2.8466	435.55	0.0096	HOMO-1 → LUMO+1 HOMO → LUMO+1	0.57049 0.41003	
3.8250	324.14	0.0380	HOMO-6 \rightarrow LUMO	0.66624	
3.9354	315.05	0.0814	HOMO-8 \rightarrow LUMO HOMO-10 \rightarrow LUMO HOMO-6 \rightarrow LUMO	0.27099 0.31063 0.16834	
3.9693	312.35	0.2638	HOMO-10 \rightarrow LUMO HOMO-8 \rightarrow LUMO HOMO-3 \rightarrow LUMO+1	0.11519 0.10562 0.50492	312
4.1045	302.07	0.2195	HOMO-3 → LUMO+1 HOMO-9 → LUMO	0.37133 0.43720	
4.1556	298.36	0.0341	HOMO-1 \rightarrow LUMO+3 HOMO-1 \rightarrow LUMO+4 HOMO \rightarrow LUMO+3 HOMO \rightarrow LUMO+4	0.28220 0.18404 0.59086 0.10021	
4.1750	296.97	0.0113	HOMO-1 \rightarrow LUMO+3 HOMO \rightarrow LUMO+4	0.60401 0.16972	

Table S5 Vertical excitation energies (E_{ex}), oscillator strengths (f), and Key transitions of thelowest few excited singlets obtained from TDDFT calculations of L in H2O

E _{excitation} (eV)	$\lambda_{\text{excitation}}$ (nm)	Osc. strength (f)	Key transitions	CI	λ _{expt.} (nm)
2.4197	512.39	0.0641	HOMO \rightarrow LUMO+1 HOMO \rightarrow LUMO+2 HOMO \rightarrow LUMO+3 HOMO-2 \rightarrow LUMO	0.21699 0.35494 0.41567 0.37931	525
2.5758	481.33	0.1323	$\rm HOMO \rightarrow \rm LUMO+2$	0.56318	
2.6046	476.02	0.7915	$HOMO \rightarrow LUMO+1$	0.61194	
2.7861	445.01	0.0220	$HOMO \rightarrow LUMO+4$	0.70195	
3.1297	396.16	0.0242	HOMO-3 \rightarrow LUMO+1 HOMO-1 \rightarrow LUMO+1 HOMO-1 \rightarrow LUMO+3	0.19382 0.65063 0.14969	
3.5752	346.79	0.0319	HOMO-6 \rightarrow LUMO HOMO-5 \rightarrow LUMO HOMO-1 \rightarrow LUMO+4	0.51980 0.29607 0.17980	
3.6526	339.44	0.0766	HOMO-2 \rightarrow LUMO+3	0.66251	
3.6721	337.64	0.2319	HOMO-6 \rightarrow LUMO HOMO-3 \rightarrow LUMO+1 HOMO-2 \rightarrow LUMO+3	0.12996 0.43916 0.23093	312

Table S6 Vertical excitation energies (E_{ex}), oscillator strengths (f), and Key transitions of the lowest few excited singlets obtained from TDDFT calculations of L'-Al Complex in H₂O