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A bright red-emitting flavonoid for Al^{3+} detection in live cells without quenching ICT fluorescence

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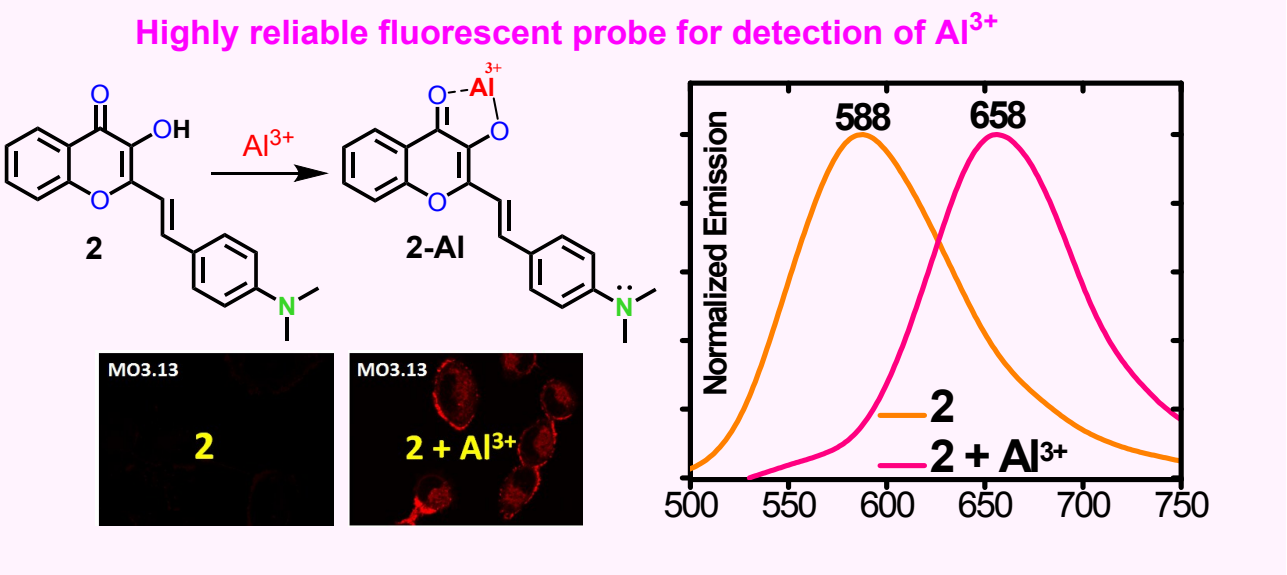

Supporting Information

Materials and Methods

All solvents and reagents were used as received without further purification. Reactions were performed under standard atmosphere condition in oven-dried glassware. All molecular biology grade reagents for cell culture and fluorescent confocal microscopy were purchased from Fisher Scientific. UV-vis spectra were obtained with Hewlett Packard-8453 diode array spectrophotometer at 25 ^{o}C. Fluorescence spectra were measured with HORIBA Fluoromax-4 spectrofluorometer. ^{1}H NMR spectra were obtained on a Varian 300 MHz spectrometer in deuterated dimethyl sulfoxide (*d*-DMSO). Fluorescence confocal laser microscopy Imaging was performed in Nikon A1 system with 60x or 100x oil objective.

Synthesis of (*E*)-2-(4-(dimethylamino)styryl)-3-hydroxy-4*H*-chromen-4-on (2)

In a 25 mL round bottom flask, 1.05 mmol of 4-(Dimethylamino)cinnamaldehyde was dissolved in 15 mL of methanol and 5 mmol of aqueous KOH was added. Then 2-Hydroxyacetophenone (1.0 mmol) was added and the resulting dark red solution was heated up to reflux for 3 hours. Upon completion, the red-orange color solution was then cooled down again to 50^{o} C and to the resulting red-orange solution, aqueous H_2O_2 solution (1 mL of 30%) was slowly added followed by 3 mmol of aqueous KOH. After 6 hours, the reaction mixture was neutralized on ice and probe **2** was collected under vacuum filtration as a red color solid. Crude product of **2** was recrystallized from hot methanol to obtain bright red powder (72%).

(*E*)-2-(4-(dimethylamino)styryl)-3-hydroxy-4*H*-chromen-4-on (2) ^{1}H NMR (DMSO-*d*, 300 MHz) δ 9.34 (s, 1H), δ 8.07 (d, 1H), δ 7.72 (d, 3H), δ 7.48 (dd, 3H), δ 7.11 (d, 1H), δ 6.75 (d, 2H), and δ

2.97 (s, 6H). ^{13}C NMR (DMSO-*d*, 500 MHz) δ 40.6 (2C), δ 110.47 (2C), δ 112.57 (1C), δ 117.99 (1C), δ 122.33 (1C), δ 123.68 (1C), δ 124.46 (1C), δ 125.03 (1C), δ 129.03 (2C), δ 133.09 (1C), δ 134.20 (1C), δ 137.09 (1C), δ 147.99 (1C), δ 151.17 (1C), δ 154.44 (1C), and δ 171.69 (1C). HRMS (ESI) found (m/z) for $[MH]^+$ 308.1211, 309.1356 and 310.1389. Calculated (m/z) for $[MH]^+$ were 308.1287, 309.1287 and 310.1287. Melting point = 245 ^{o}C – 248 ^{o}C.

Fluorescence Quantum yield

The fluorescence quantum yields (ϕ_{fl}) for compounds were calculated by using Rhodamine 6G (sigma) as the standard (ϕ_{ref} = 0.95, ethanol) at 490 nm. The following equation was used for calculation**.**

$$(\phi_{fl})sample = \phi_{Ref} x \frac{Abs_{Ref}}{Abs_{Sample}} x \frac{I_{Sample}}{I_{Ref}} x \frac{(\eta_{Ref})^2}{(\eta_{Sample})^2}$$

where, Abs is the absorbance of the sample, I is the integrated fluorescence emission intensity and η is the refractive index of the solvent.

Spectrometric titration with Al^{3+}

Spectrometric titrations were conducted in water: acetonitrile (10:90) mixture at room temperature. 1 mM Al^{3+} solutions were prepared by using $Al(NO_3)_3$.9H2O solution (in ultra-purified water). All spectrometric titrations were triplicated in order to assure the consistency of the results. The binding constant (K) for **2-Al** complex was calculated by using the emission of the complex at 658 nm according to following equation.

$$\log \{ [I - I_0] / [I_{max} - I] = \log K + n \log [Al^{3+}] \}$$

Where I_{max} is the maximum fluorescence intensity obtained for **2-Al** complex and I_0 is the fluorescence intensity observed in the absence of Al3+ in the media. Where I is the fluorescence intensity recorded in the presence of Al^{3+} in the media.

Cell culturing, staining and live cell imaging.

Fluorescent confocal microscopy imaging was performed on a Nikon A1 confocal system with 60 x and 100 × oil objective with numerical aperture of 1.45 and refractive index of 1.5. Imaging temperature was maintained at 37^{o} C at all time. The excitation for **2** was 488 nm. Standard DAPI, FITC, and mCherry emission filters were used. All imaging was done in an Okolab Bold Cage Incubate or at 37^{o}C, and images were processed using NIS Elements or ImageJ Pro (NIH) imaging software.

MO3.13 cells (progenitor oligodendrocytes) cells were plated on Mat Tek 35 mm dish with glass bottom at a density of 1x10^{5} cells per plate in DMEM media enhanced with 10% fetal bovine serum (Gibco) and 1% penicillin/streptomycin. Cells were incubated overnight at 37^{o}C and in 5% CO_2. Subsequent to incubation, cells were washed with 1× PBS and treated with 1 μM stain in Invitrogen™ Molecular Probes™ Live Cell Imaging Solution for 30 min. After treatment, cells were washed once for 1 minute with 1 × PBS. Invitrogen™ Molecular Probes™ Live Cell Imaging Solution was added to cells for imaging. For Al^{3+} analysis experiments cells were pre incubated with 1 μM $Al(NO_3)_3$.9H2O solution (in water) for 30 minutes prior to treatment of probe **2**. All cells were maintained in a 5% CO_2 humidified atmosphere at 37°C.

Cell viability assay

The viability of NHLF following treatment with Probe 1 was evaluated using Alamar Blue assay. NHLF were plated in a 96 well plate at 8 x 104 cells/mL with DMEM 10% FBS and 1% penicillin/streptomycin and allowed to adhere overnight at 37°C with 5% CO2. Probe treatments, 0.1 μM - 100 μM, or vehicle control, 0.5% DMSO, were dissolved in DMEM 10% FBS and 1%

penicillin/streptomycin. Each treatment concentration was applied in triplicate, except for vehicle control that was applied to 12 wells and incubated for 24 hours. After24 hours, media and treatment were aspirated and replaced with clear media with 10% FBS and 1% penicillin/streptomycin containing 10% of the solution resazurin and incubated for 3.5 hours at 37°C with an atmosphere of 5% CO2 and 95% humidity. The culture plate was wrapped in aluminum foil while incubating. Read fluorescence at excitation 530–570 nm and emission 580–620 nm wavelength on a plate reader. Percent viability was determined by the following (treatment absorbance-average value for no cell control) / (average vehicle control absorbance – average value for no cell control) × 100. Dose-response curves and statistics were generated using GraphPad Prism 5 software.

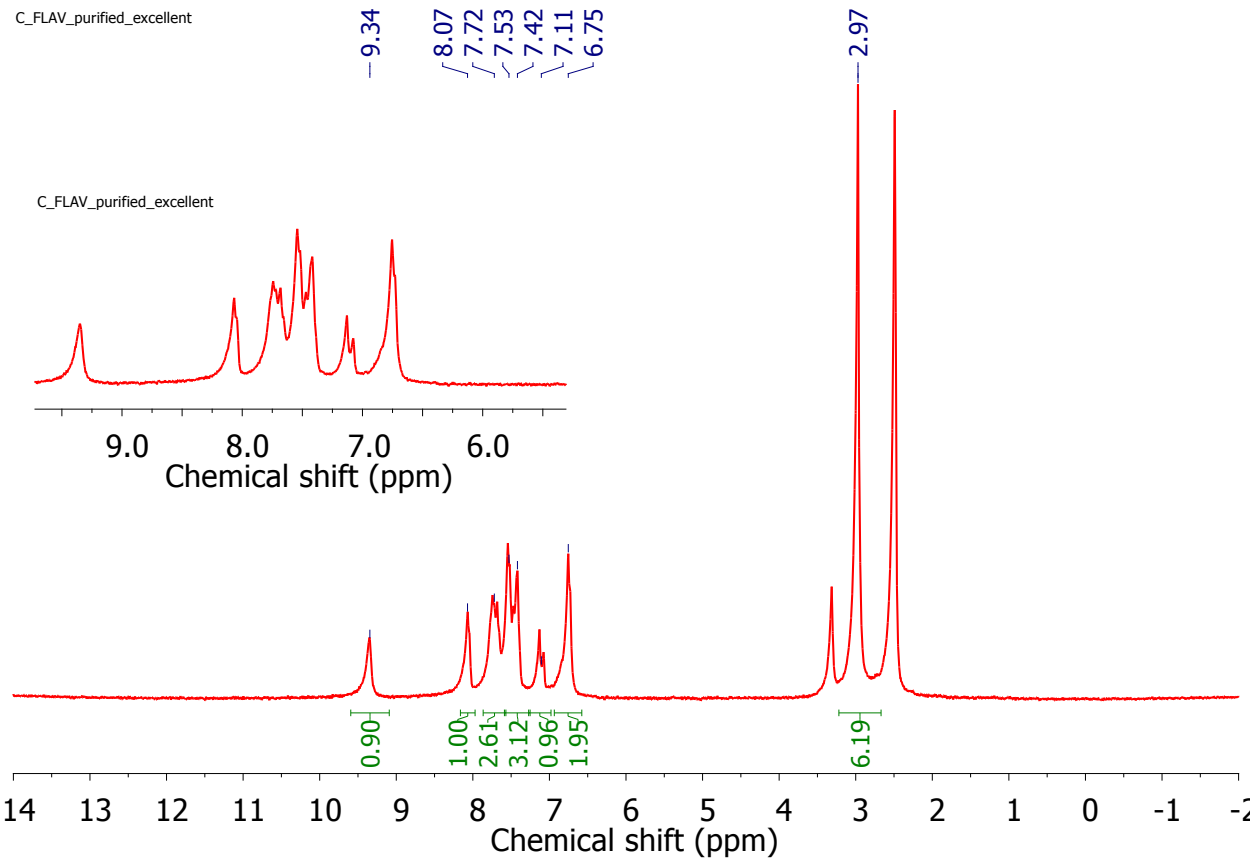

Figure S1.1 ^{1}H NMR spectra of **2** in DMSO-d6 (300 MHz). The response signals at 2.5 ppm and 3.3 ppm attributed to DMSO and water respectively.

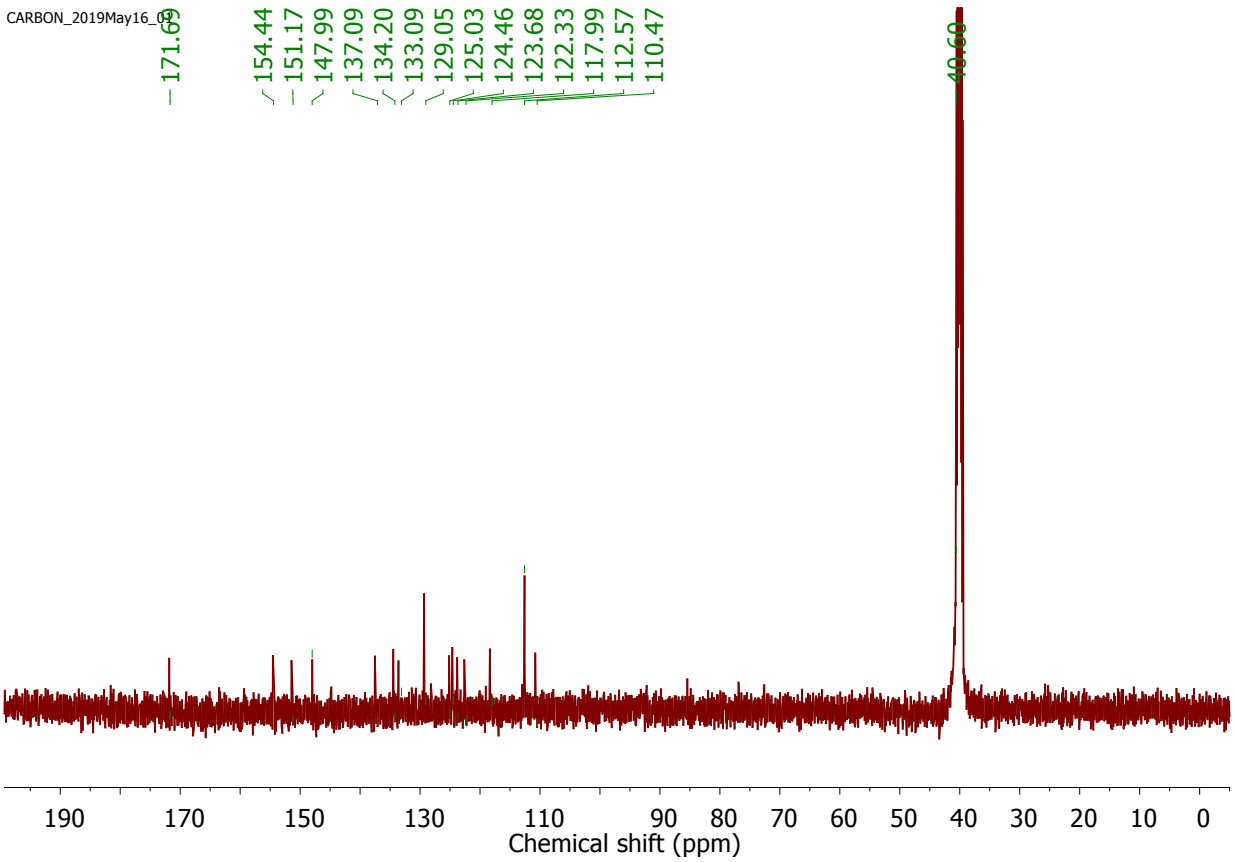

Figure S1.2 ^{13}C NMR spectra of **2** in DMSO-d6 (500 MHz).

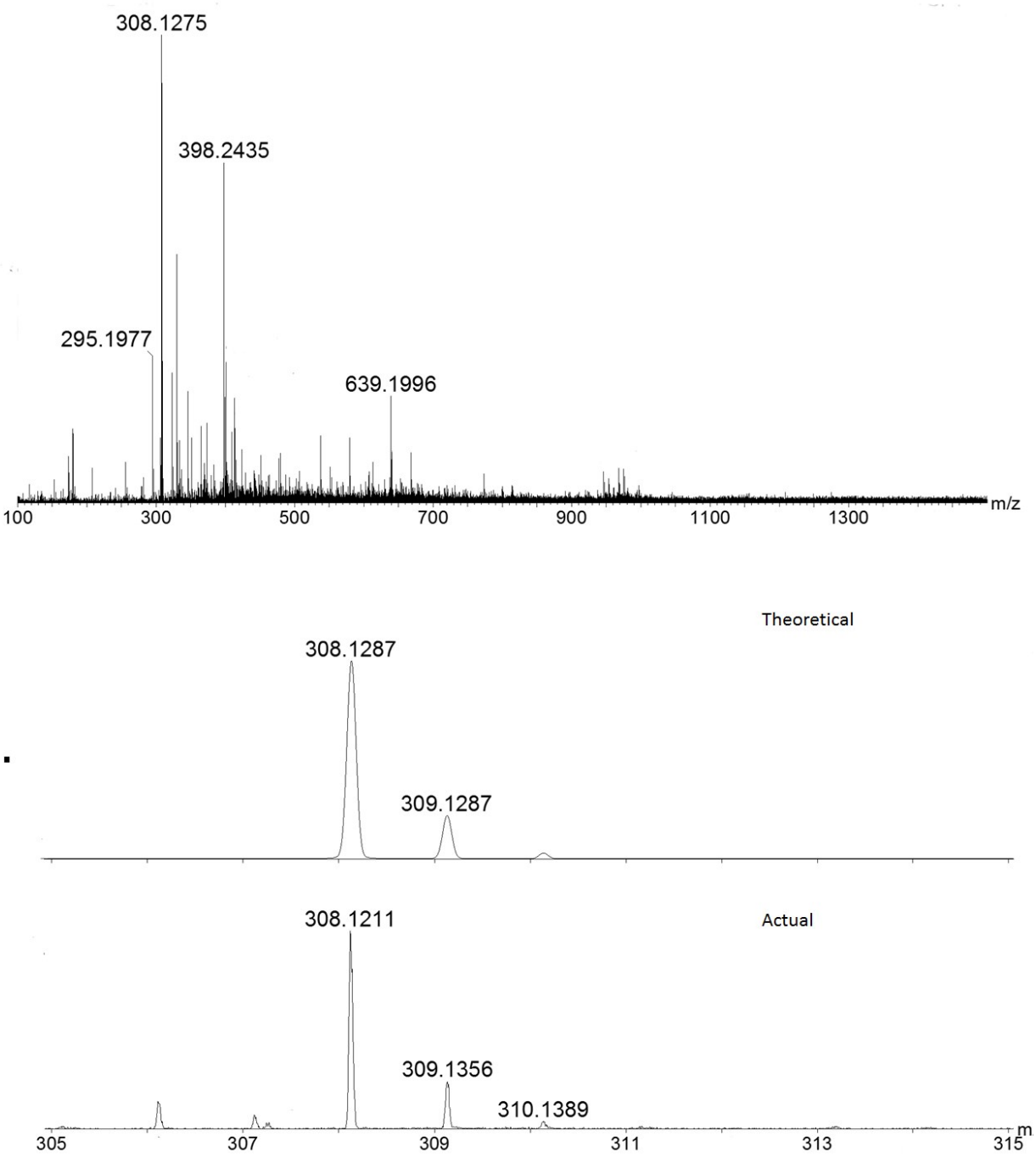

Figure S2. High resolution mass spectra (ESI) of [**2**-H]$^{+}$. (Error < 3ppm)

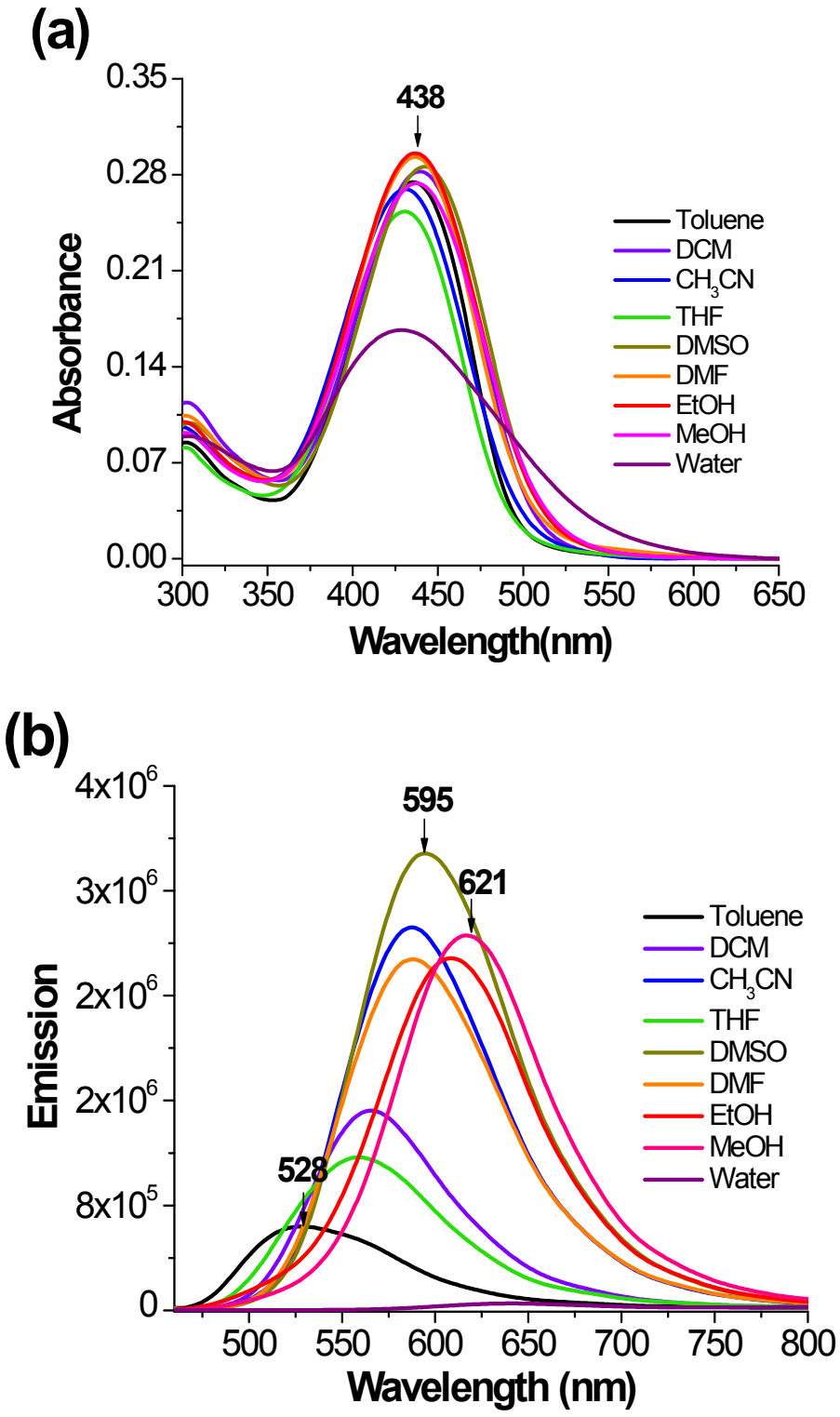

Figure S3. Absorption (a) and emission (b) of probe **2** (1 x 10^{-5} M) in different solvents at room temperature.

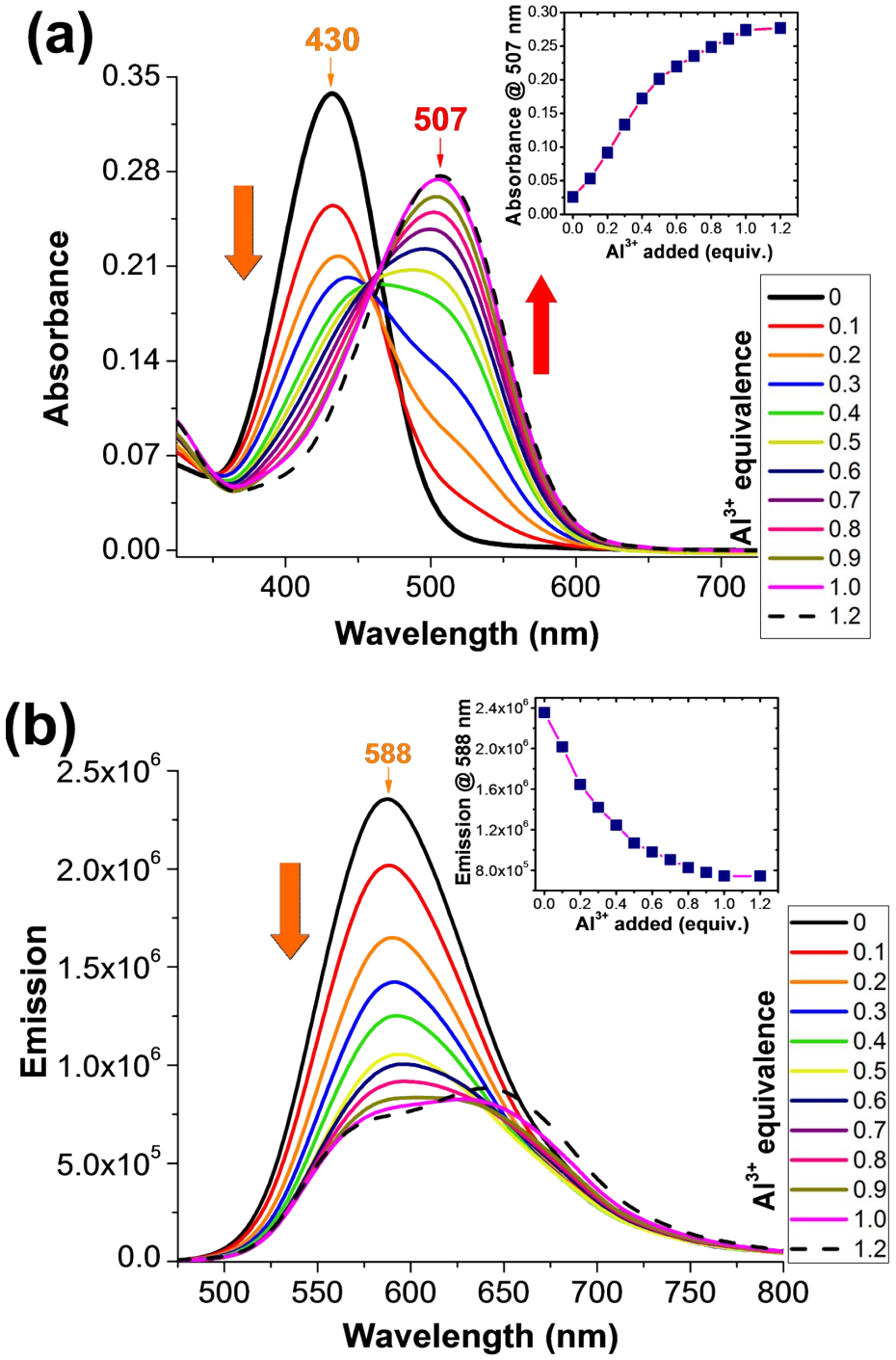

Figure S4. Absorption (a) and emission (b) recorded for probe **2** (1 x 10^{-5} M) in acetonitrile upon spectrometric titration with Al^{3+} (1 mM in water) at room temperature. Sample was excited at 430 nm to obtain emission spectra.

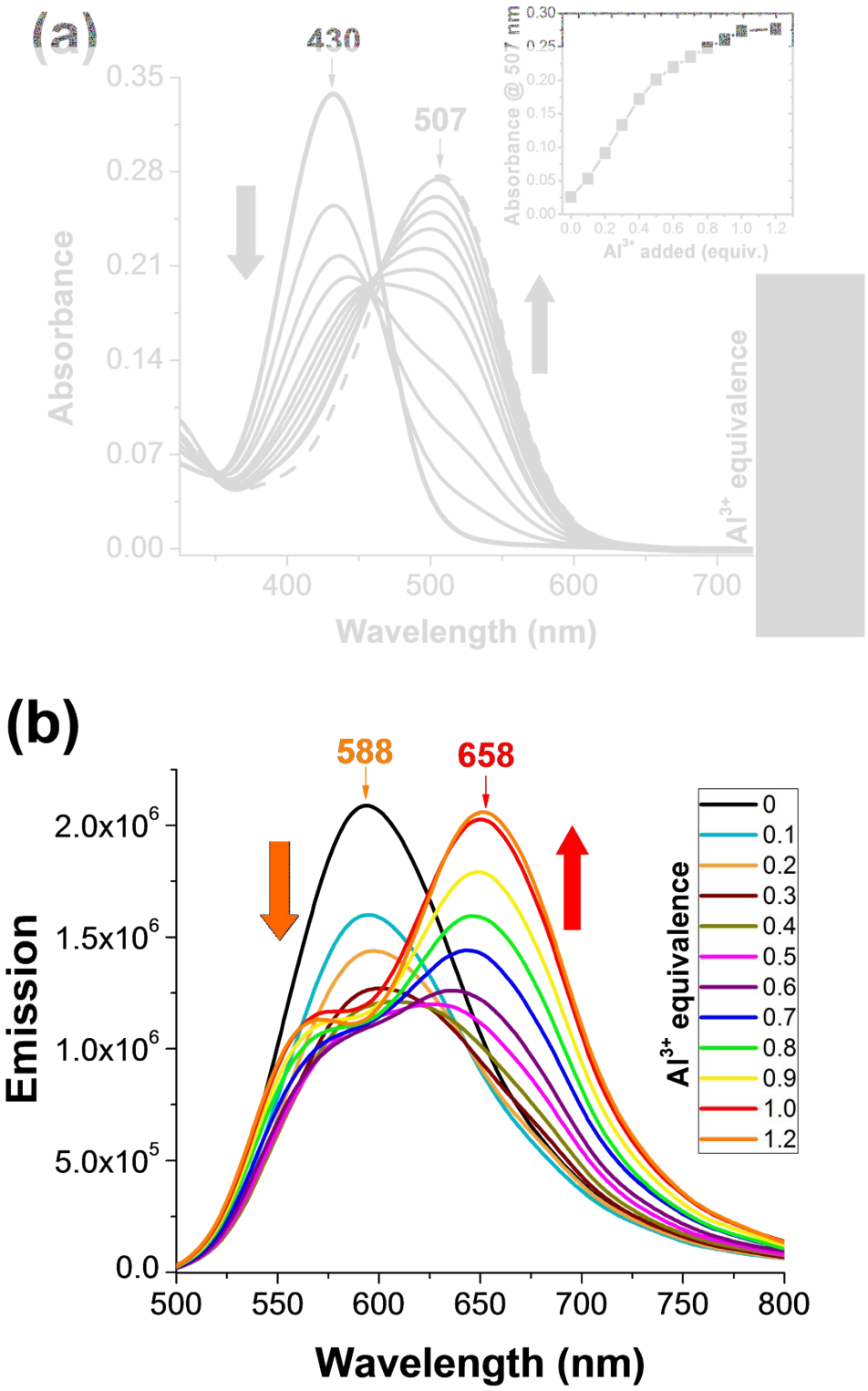

Figure S5. Absorption (a) and emission (b) recorded for probe **2** (1 x 10^{-5} M) in acetonitrile upon spectrometric titration with Al^{3+} (1 mM in water) at room temperature. Sample was excited at 465 nm to obtain emission spectra.

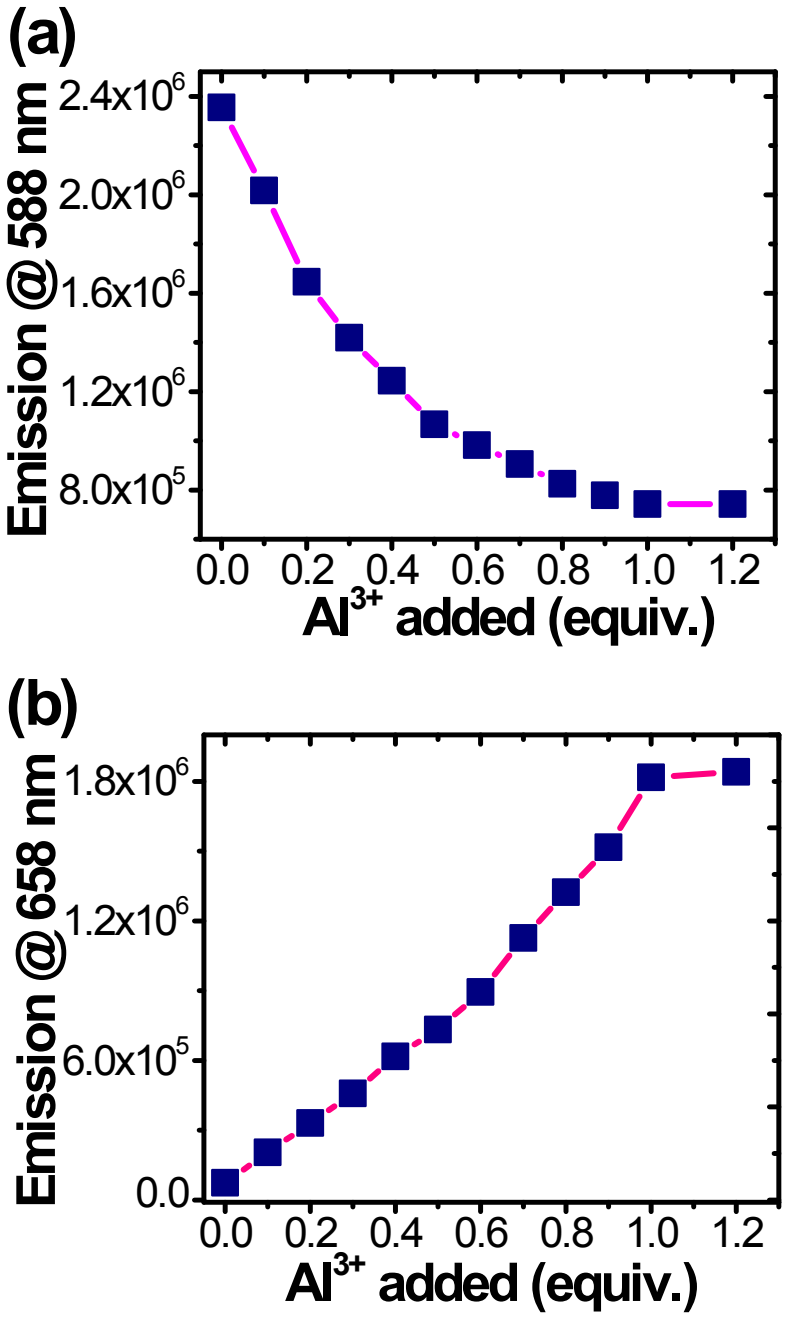

Figure S6. Emission spectra obtained for probe **2** (1 x 10^{-5} M) in acetonitrile at 588 nm (a) and 658 nm (b) upon sequential addition of A^{3+}.

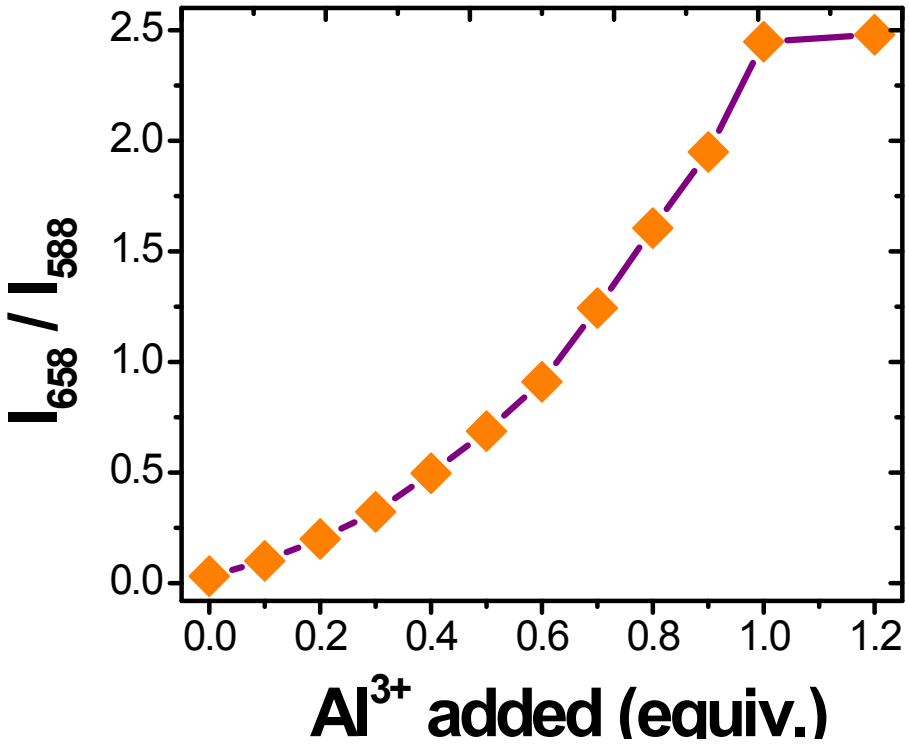

Figure S7. Ratiometric determination of the emission intensities obtained for probe **2** at 588 nm and 658 nm upon sequential addition of Al^{3+} in to the solution.

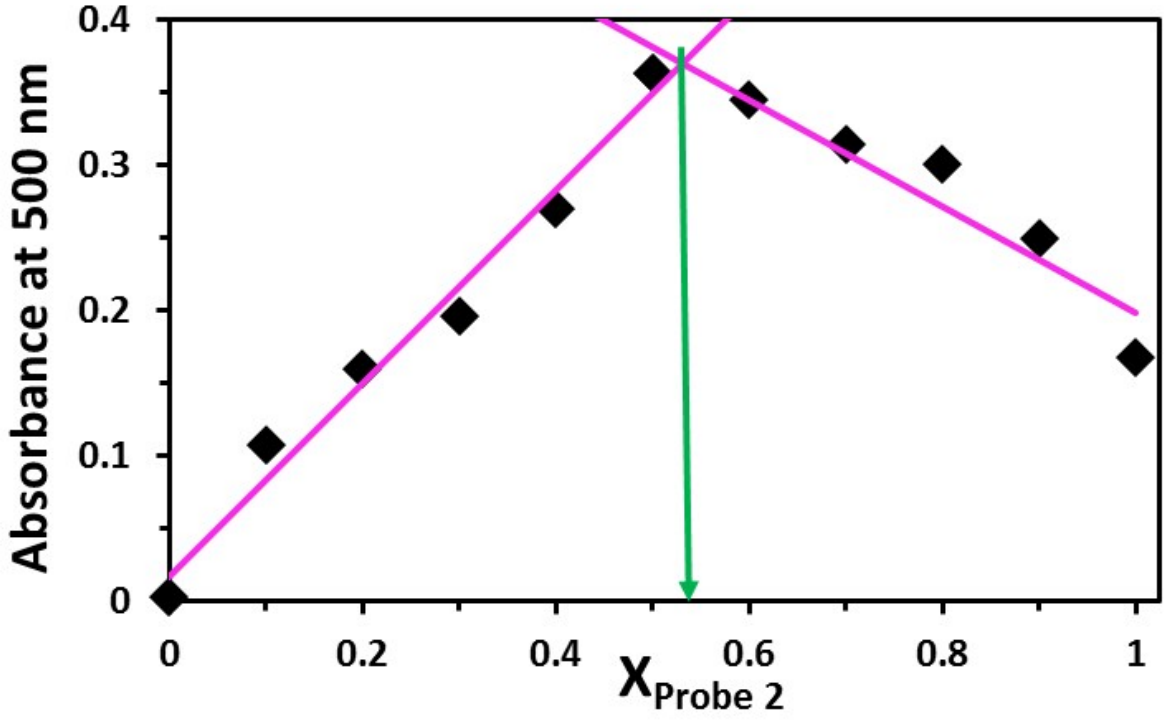

Figure S8. Determination of the probe **2**: Al^{3+} binding stoichiometry by Job's method.

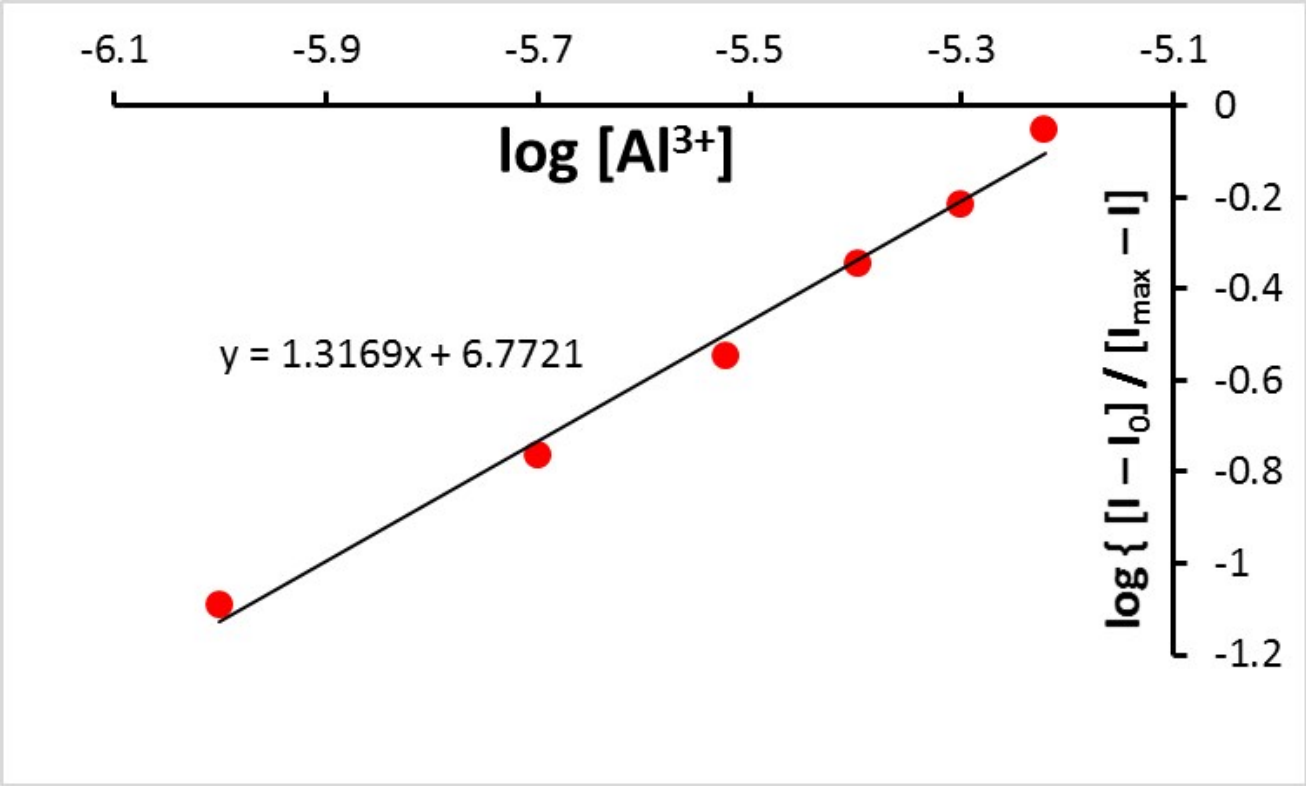

Figure S9. Determination of the binding constant (log K) for **2-Al** complex by using emission data obtained during spectrometric titration of probe **2** with Al^{3+}.

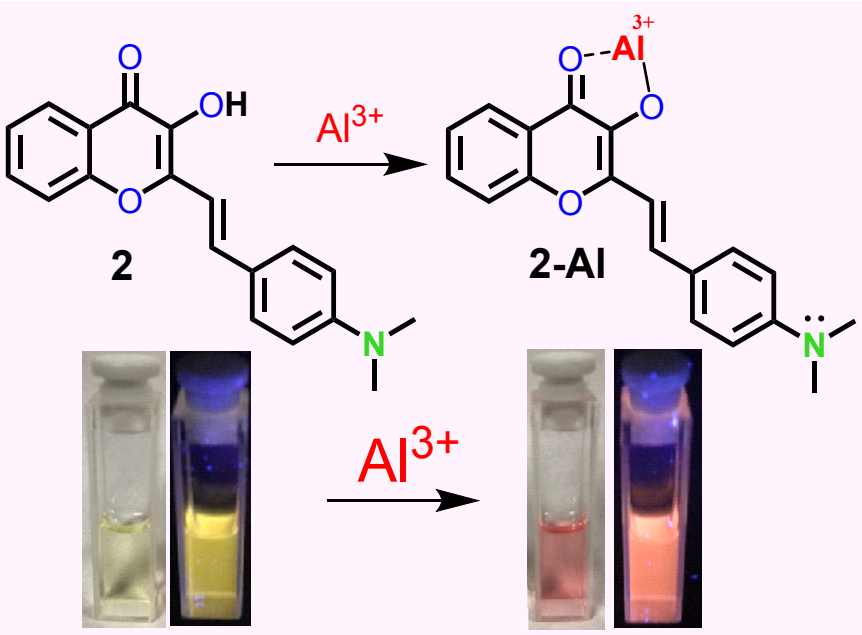

Figure S10. Visible and UV illuminated color observed for probe **2** before (left) and after (right) the addition of Al^{3+} (1.0 equiv.) in acetonitrile at room temperature.

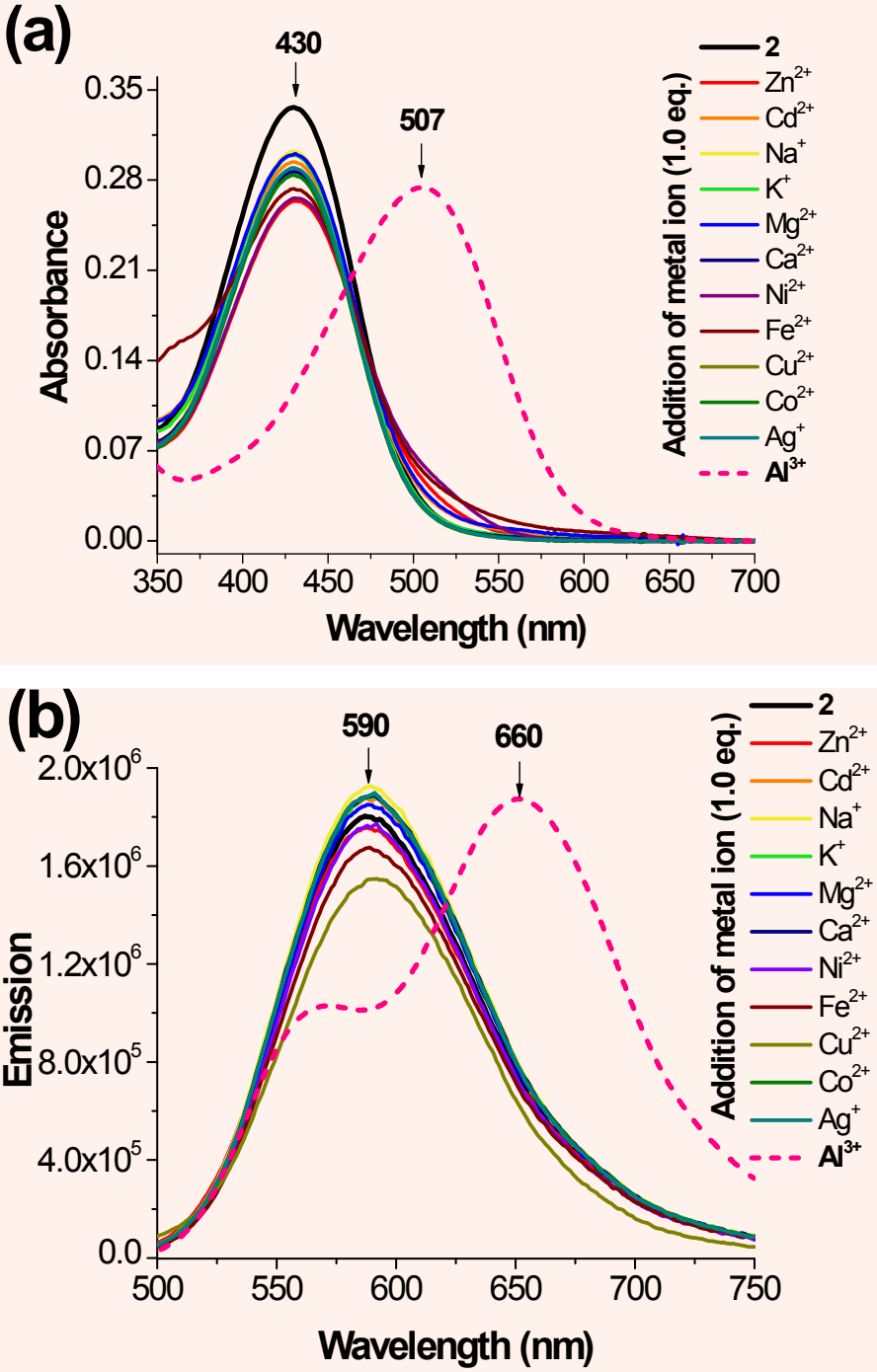

Figure S11. Absorption (a) and emission (b) recorded for probe **2** (1 x 10^{-5} M) in acetonitrile upon addition of 1 eq. of cationic species (1 mM in water) at room temperature. Only **2**-**Al** complex was excited at 465 nm and all other samples were excited at 430 nm.

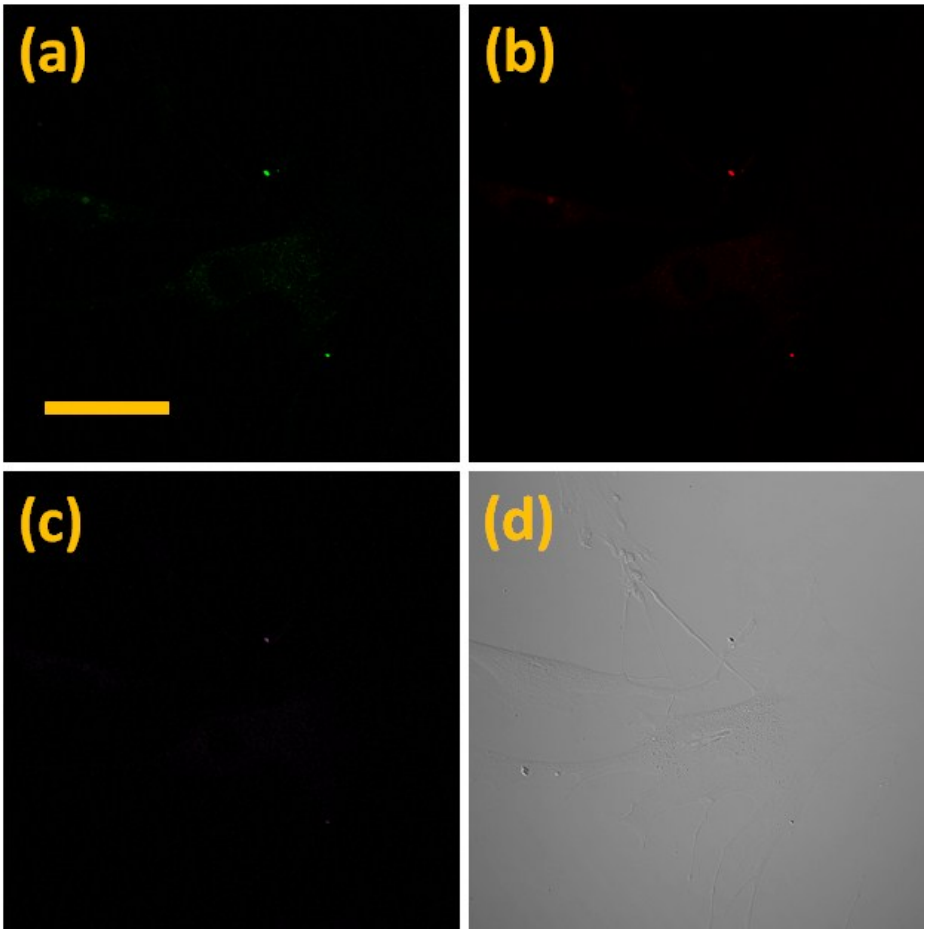

Figure S12. Fluorescence confocal microscopy images obtained for MO3.13 cells incubated with probe **2** (1 µM) for 30 minutes. Images shows (a) green channel (500 – 575 nm, (b) red channel (565 – 700 nm), (c) cyanine channel (710 – 790 nm), and (d) bright field imaging. Probe **2** was excited with 488 nm laser.

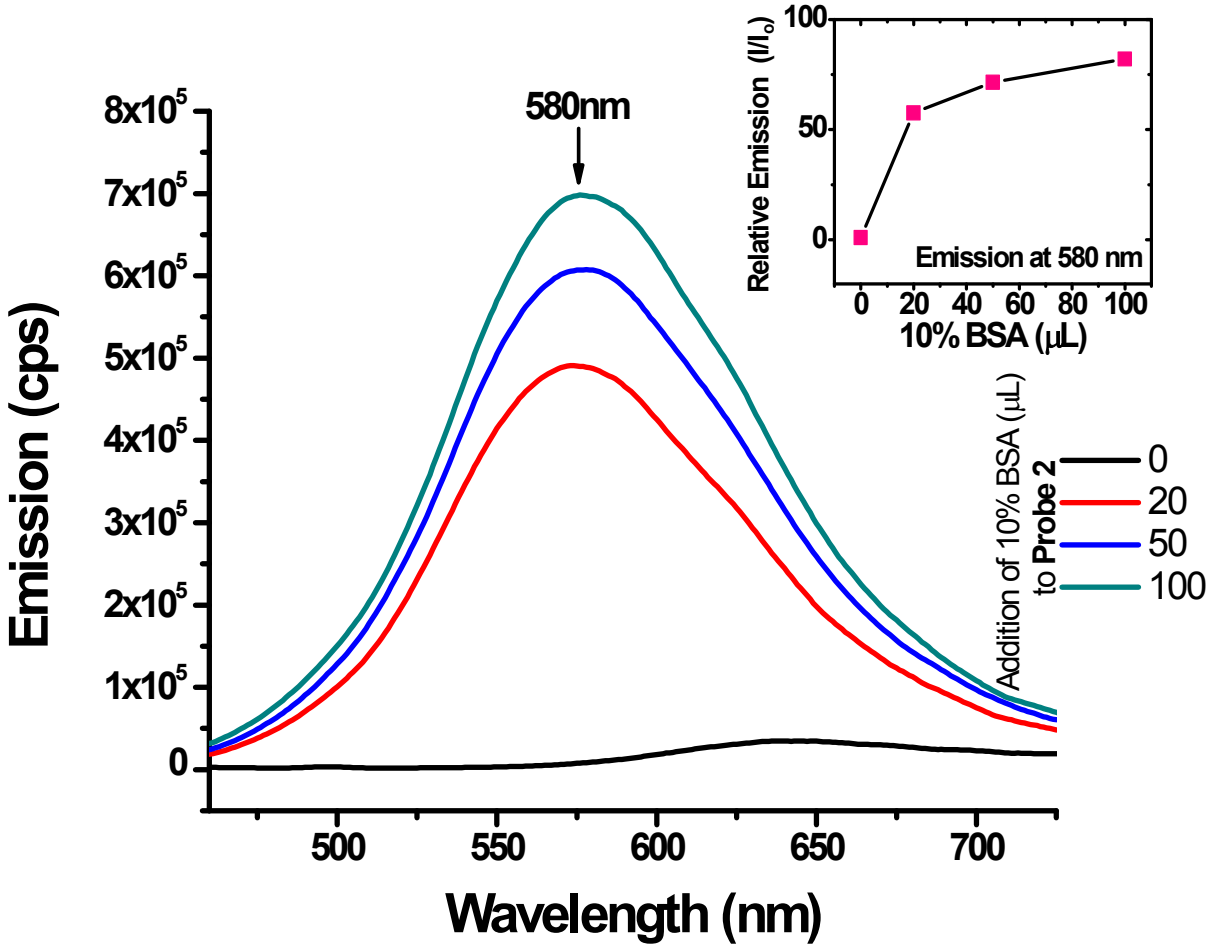

Figure S13. Fluorescence emission spectra obtained for probe **2** (1 x 10^{-5} M in water) upon addition of different amounts of 10% (w/v) BSA in water. Probe **2** was excited at 435 nm to obtain the emission spectra

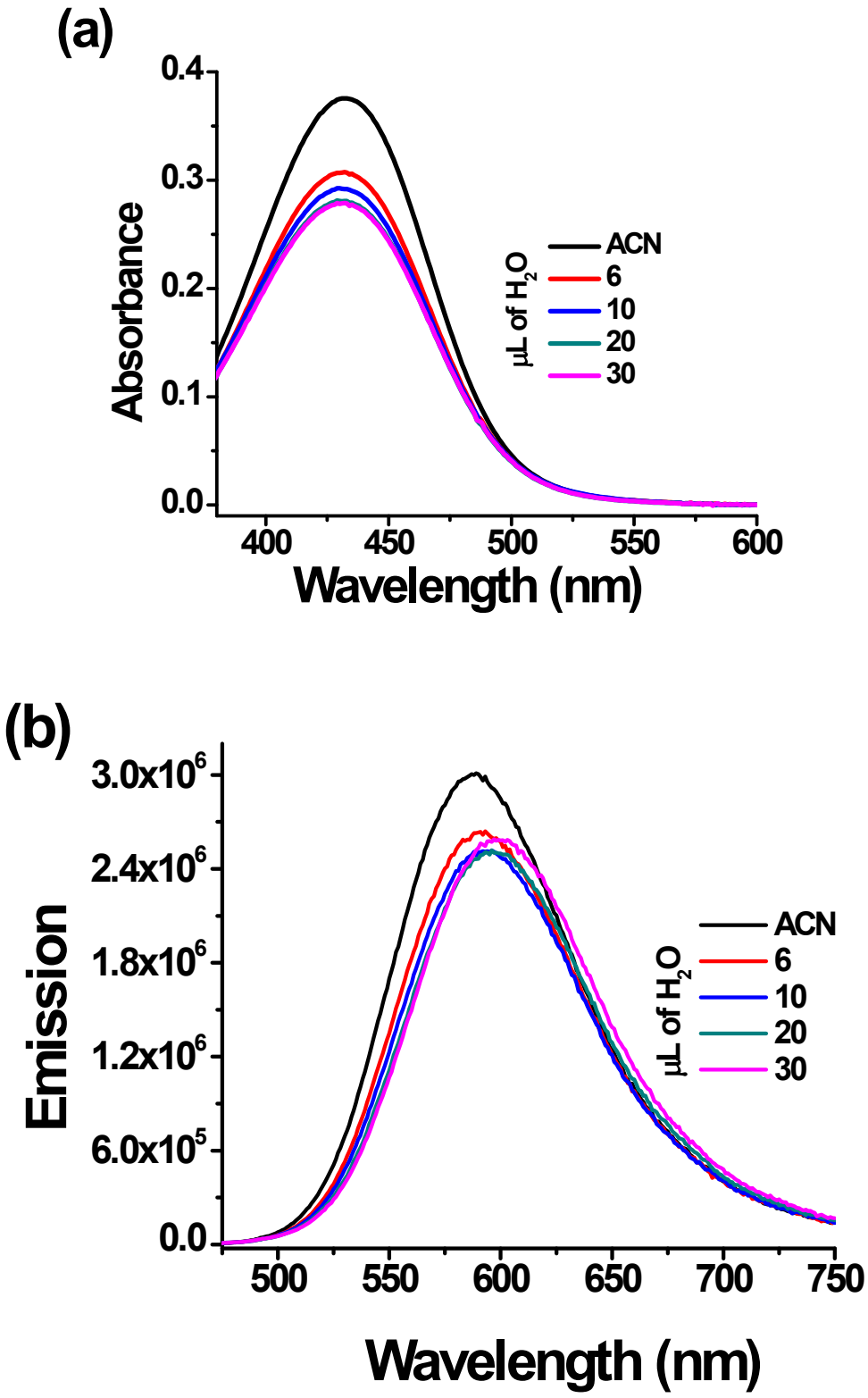

Figure S14. Absorbance (a) fluorescence (b) spectra obtained for probe **2** (1 x 10-5 M) in acetonitrile at room temperature upon addition of different amounts of water. Probe **2** was excited at 430 nm to obtain the emission spectra.

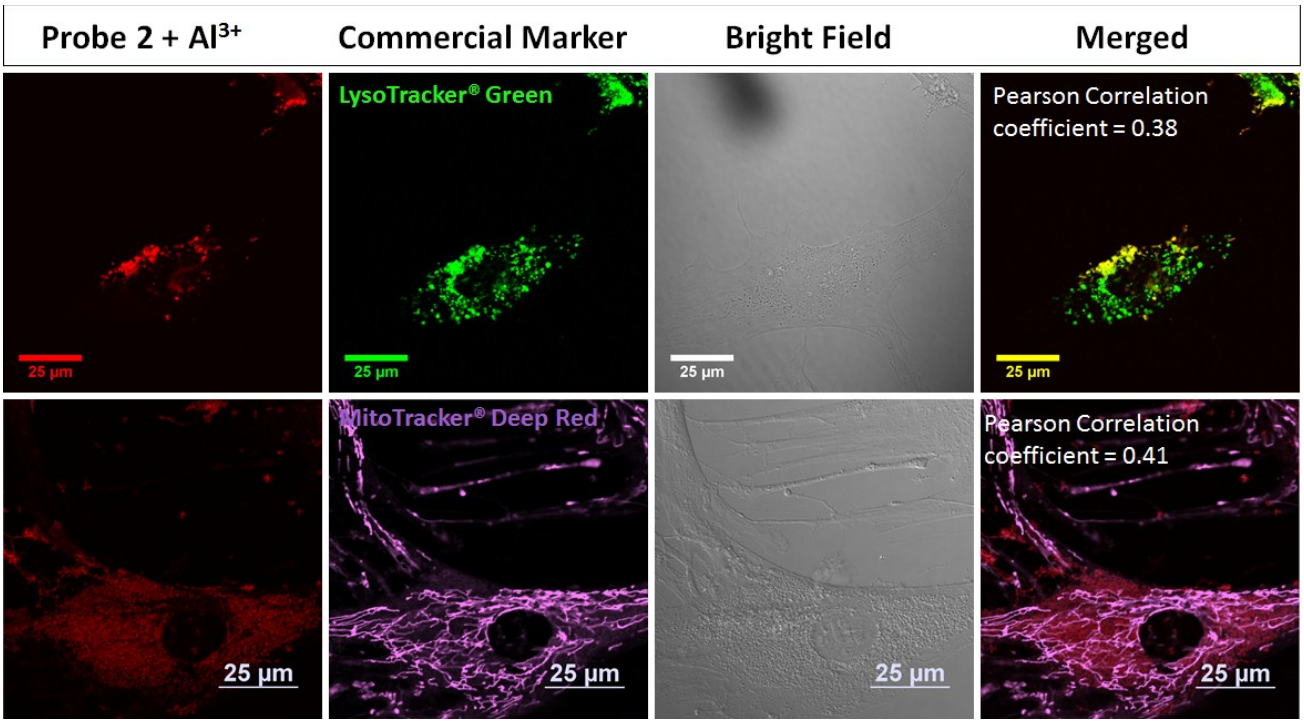

Figure S15. Absorbance Colocalization experiments carried out for probe **2** +**Al** (1 µM) complex in the presence of LysoTracker® Green (100 nm) and MitoTracker® Deep Red (200 nM) in NHLF Cells. Probe **2** + **Al** complex was excited with 561 nm laser line, MitoTracker® Deep Red was excited with 640 nm laser line and LysoTracker® Green was excited with 488 nm Laser Line.

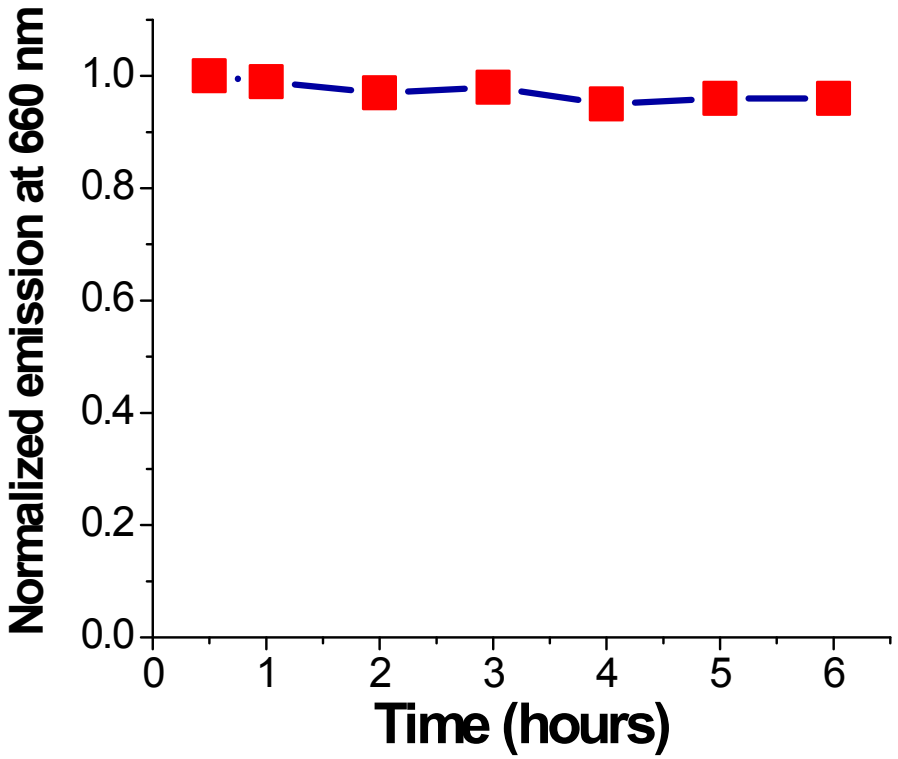

Figure S16. Normalized emission at 660 nm calculated for probe **2** + **Al** complex (10 µM in acetonitrile) at room temperature for 6 hours. The complex was excited at 507 nm to obtain the emission.

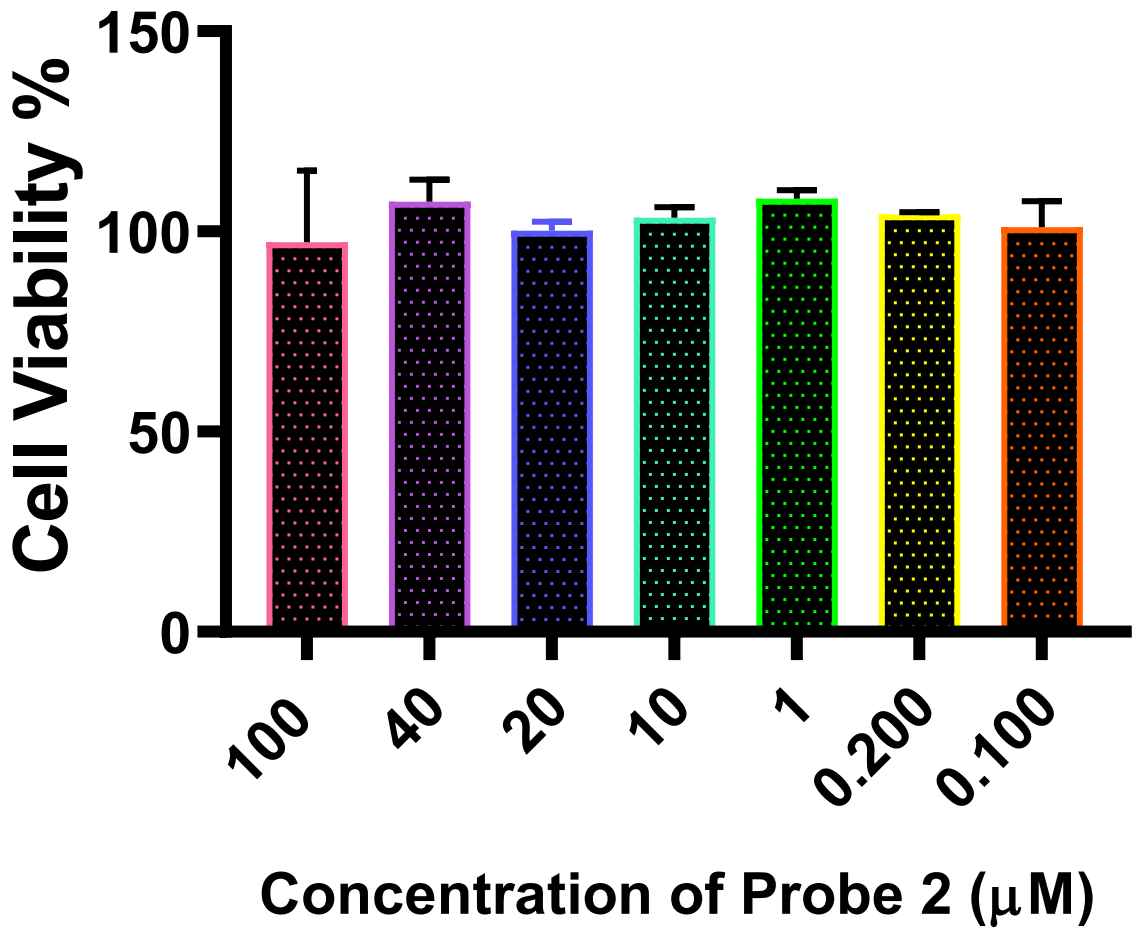

Figure S17. Resozurin based cell viability assay conducted for probe **2** in NHLF cells.

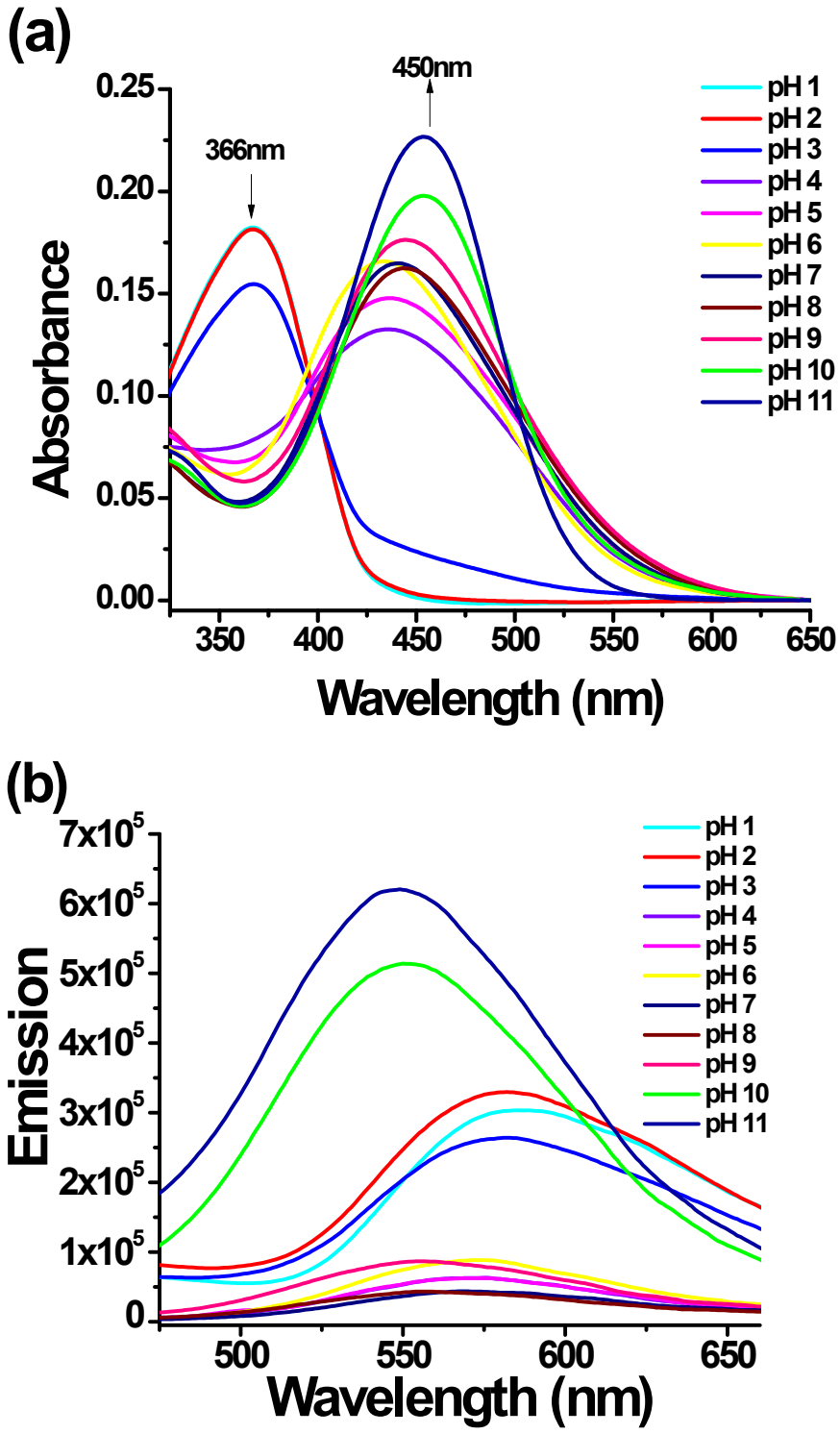

Figure S18. Absorbance (a) and emission (b) of probe **2** in different aqueous pH solutions at room temperature. Excitation at 450 nm.

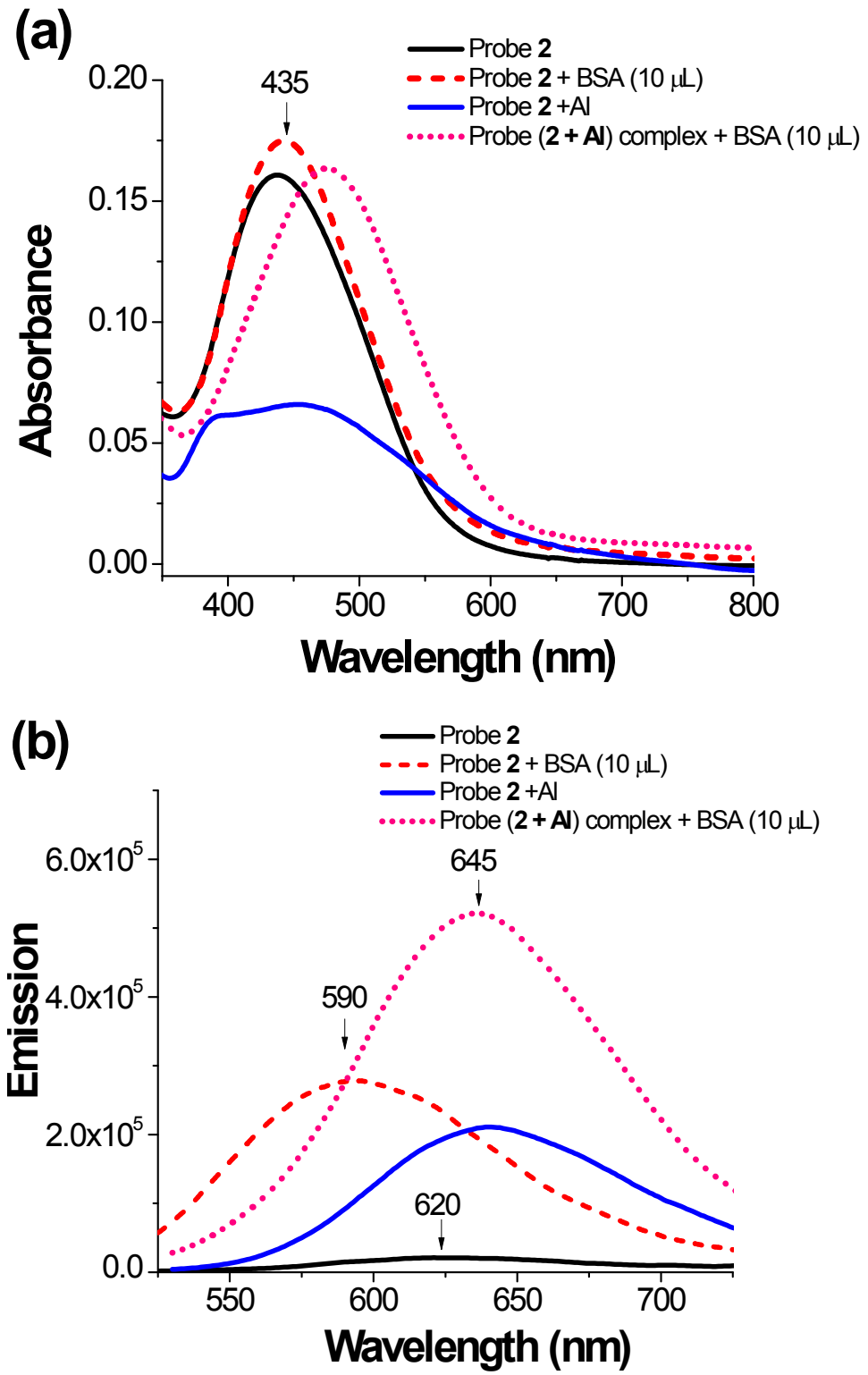

Figure S19. Absorbance (a) and emission (b) obtained for Probe **2** and **2+Al** complex in aqueous buffer (pH = 7) upon addition of 10% BSA at room temperature.

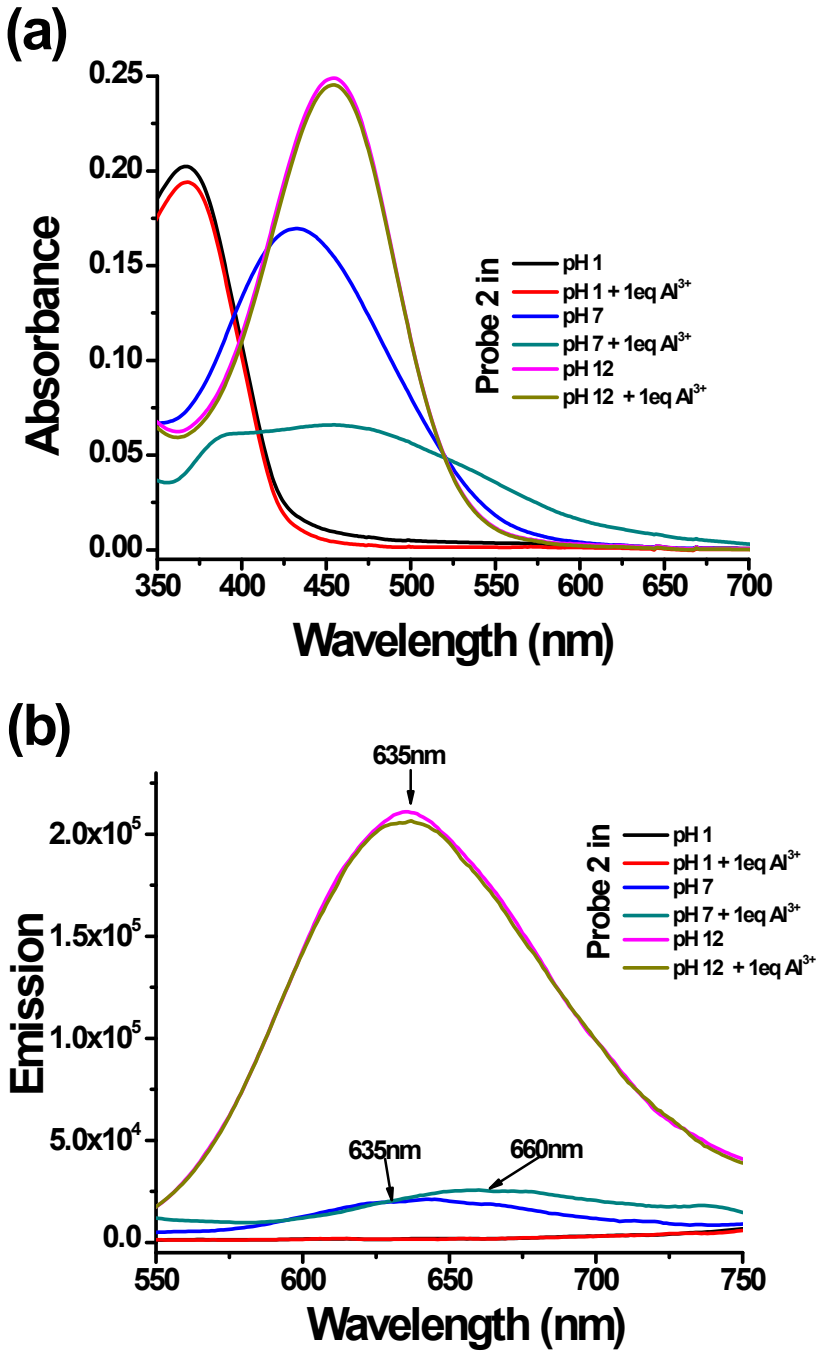

Figure S20. Absorbance (a) and emission (b) of probe **2** and probe **2+Al** complex in different aqueous pH solutions at room temperature.