Supporting information

Asymmetric Schiff Base - Based Colorimetric and Fluorescent Sensor for Al^{3+} Detection in Real Samples and Live-Cell Bioimaging

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Instruments

All the solvents and reagents (analytical grade and spectroscopic grade) were purchased from Sigma Aldrich and used as received. Stock solutions of AMMN and metal salts were prepared in a mixed solvent consisting of DMSO and HEPES in DMSO/ HEPES (1:9 v/v, pH 7.4) and subsequently diluted with the same solvent as needed for testing. Elemental analysis for carbon, nitrogen, and hydrogen was performed using a PerkinElmer 2400 CHN elemental analyzer at in the Microanalytical Unit (MAU), Cairo University, Egypt. FT-IR spectra were recorded using KBr discs covering the range of 4000–400 cm^{-1} on a Unicam-Mattson 1000 FT-IR at the Central Lab Unit, Faculty of Science, Ain Shams University, Egypt. ^{1}H and ^{13}C NMR spectra were recorded at the Microanalytical Unit (MAU) using a Bruker instrument (Munich, Germany) with DMSO-d_6 as the solvent and tetramethylsilane (TMS) as the internal standard. Chemical shifts were reported in ppm on the δ scale. Electrospray ionization mass spectrometry (ESI-MS) analysis was conducted in positive ion mode using a liquid chromatography ion trap mass spectrometer (LCQ83 Fleet, Thermo Fisher Instruments, USA) at the Centre of Excellence for Drug Research and Pharmaceutical Industries, King Abdulaziz University, Saudi Arabia. UV-visible spectra were obtained using a Shimadzu UV-Vis 1800 spectrophotometer. Luminescence measurements were carried out on a Jenway 6270 fluorimeter with a 1-cm path length quartz cuvette at 25 °C, employing a pulsed xenon lamp as the excitation source. UV-vis and fluorescence spectra were recorded in quartz cuvettes at room temperature. The time-resolved photoluminescence measurements were performed by Horiba Fluorolog-QM with DeltaFlex TCSPC system at the Ultra-Fast Dynamic Spectroscopy Laboratory, Department of Chemistry, Faculty of Science, Ain Shams University. Confocal fluorescence images were constructed by using a scanning stage at Biomedical Physics Department, King Faisal Specialist Hospital & Research Centre.

Fluorescence Lifetime Studies

Fluorescence decay measurements were carried out using a time-correlated single-photon counting spectrometer, equipped with a photomultiplier tube (R3237 PMT) as the detector and a 375 nm LED as the excitation source. The instrument response function of the TCSPC system is approximately 540 ps. Data analysis was performed using the DAS-6 software from IBH, which applies a deconvolution technique based on nonlinear least-squares methods. The fit quality is typically assessed by the reduced χ^2 value, weighted residuals, and the autocorrelation function of the residuals.

DFT Calculation

All computations were conducted using the GAUSSIAN09W software [46]. The geometry of sensor AMMN and its Al^{3+} complex was fully optimized using the DFT method at the B3LYP level of theory in the gas phase. A 6-31G(d,p) basis set was applied for C, H, N, and O atoms, while the LANL2DZ basis set with an effective core potential was used for the Al atom. Frontier molecular orbitals (FMOs) were obtained from the optimized geometries.

Cell cytotoxicity (MTT assay) and Bioimaging

To examine the cell viability of the chemosensor AMMN, 3-(4, 5- dimethylimidazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) analysis was performed on HeLa cell lines. HeLa cells derived from cervical cancer cells were procured from Vacsera, Giza, Egypt and grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), 1% L-glutamine-penicillin-Streptomycin at 37 ^{o}C and 5% CO_2. In a well plate, the cells were taken at a density of 1.5×10^4 units. Then the setup was incubated with the chemosensor AMMN, ranges from 0 to 50 μM for 30 min. After that, 100 μM of MTT was dropped in each well and warmed at 37 °C for 4 h. During this process, MTT reacts with metabolically active cells and produces the formazan crystals. The formed crystals from each well were dissolved using 100 μl of DMSO and shaken for 10 min. Then, the absorbance of the solution was measured using a plate reader and the cell viability was the percentage of the optical density ratio of the cells in the presence of sensor AMMN to the cells untreated. The HeLa cells were incubated successively with PBS buffer containing 5 μM sensor AMMN (sensor was dissolved in DMSO/HEPES, v/v =1:9, pH = 7.4) and 5 μM Al^{3+} real time dynamic monitoring was performed every minute for single cells.

Real sample analysis

A 2 mL water sample is spiked with 50 µL, 100µL, and 250 µL for colorimetric sensing and 5 µL, 15µL, and 25 µL for fluorometric analysis of Al^{3+} ions (1 mM), followed by thorough mixing. 25 µL of AMMN (1mM) probe dissolved in DMSO/HEPES buffer (1:9 v/v, pH 7.4) was added and the total volume is completed to 5 mL by HEPES buffer 7.4. Then, the absorbance or fluorescence measurements were performed in a quartz cuvette after equilibration for 1 min. at 375 nm and 503 nm, respectively.

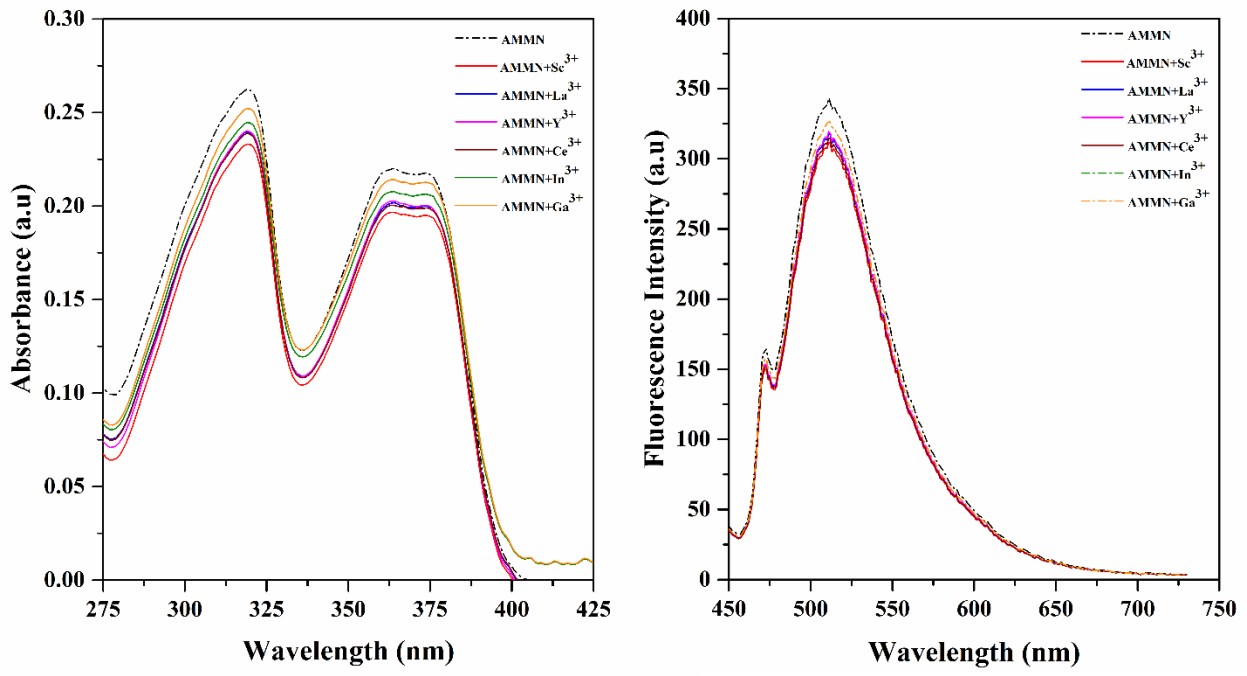

Fig. S1. Absorption and Fluorescence spectra of AMMN in presence of the trivalent ions Sc^{3+}, La^{3+}, Y^{3+}, In^{3+} and Ga^{3+}.