Electronic Supporting Information

for

Rhodamine based "Turn-On" Fluorescent Probe for Pb(II) and their live cell imaging

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Materials and methods:

All reagents and solvents were used without purification. Rhodamine 6G and trimethoxybenzaldehyde were purchased from Acros Organics and Aldrich respectively. Rhodamine 6G hydrazide was prepared by the reported procedures. NMR spectra were recorded on a Bruker (Avance) 300 MHz instrument using TMS as internal standard. ESI-MS spectral analysis was performed in positive ion mode on a liquid chromatography-ion trap mass spectrometer (LCQ Fleet, Thermo Fisher Instruments Limited, US).Metal chloride salts were used as the source for metal ions. Absorption measurements were carried out using a JASCO V-530 UV-vis spectrophotometer. Fluorescence spectra were recorded on Agilent Cary eclipse fluorescence spectrophotometer. The slit width was 5 nm for both excitation and emission.

UV-vis-fluorescence titration studies:

The absorption and fluorescence responses of the probe RDP-1 towards various metal ions was investigated by UV-vis spectroscopy and fluorescence spectroscopy respectively in phosphate buffered solution (pH 7.54) with 1 % DMSO as a co-solvent. The metal chloride salts was used as the source for metal ions.

MTT assay:

The cell viability of the probe RDP-1 were tested against HeLa cell lines using the 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. The cells were seeded into a well plate at a density of 1.5×10^4 cells per well and incubated in medium containing RDP-1 at concentrations ranging from 0- 50 μM for 48 h. To each well, 100 μL of MTT was added and the plates were incubated at 37 °C for 4 h to allow MTT to form formazan crystals by reacting with metabolically active cells. The medium with MTT was removed from the wells. Intracellular formazan crystals were dissolved by adding 100 μL of DMSO to each well and the plates were shaken for 10 min. The absorbance was recorded using Plate reader.

Cell culture and fluorescence imaging:

HeLa cells were grown in modified Eagle's medium supplemented with 10% FBS (fetal bovine serum) at 37 °C. The HeLa cells were incubated with the probe RDP-1 (10.0 μM in DMSO/H_2O (2:8, v/v) buffered with HEPES buffer) and imaged through fluorescence

microscope. The cells were washed with HEPES three times to remove the excess of the probe RDP-1 in the extra cellular parts and growth medium. Again the probe treated cells were further incubated with $PbCl_2$ (10.0 μM in H_2O) for 10 min at 37 °C and imaged with Nikon fluorescence microscope.

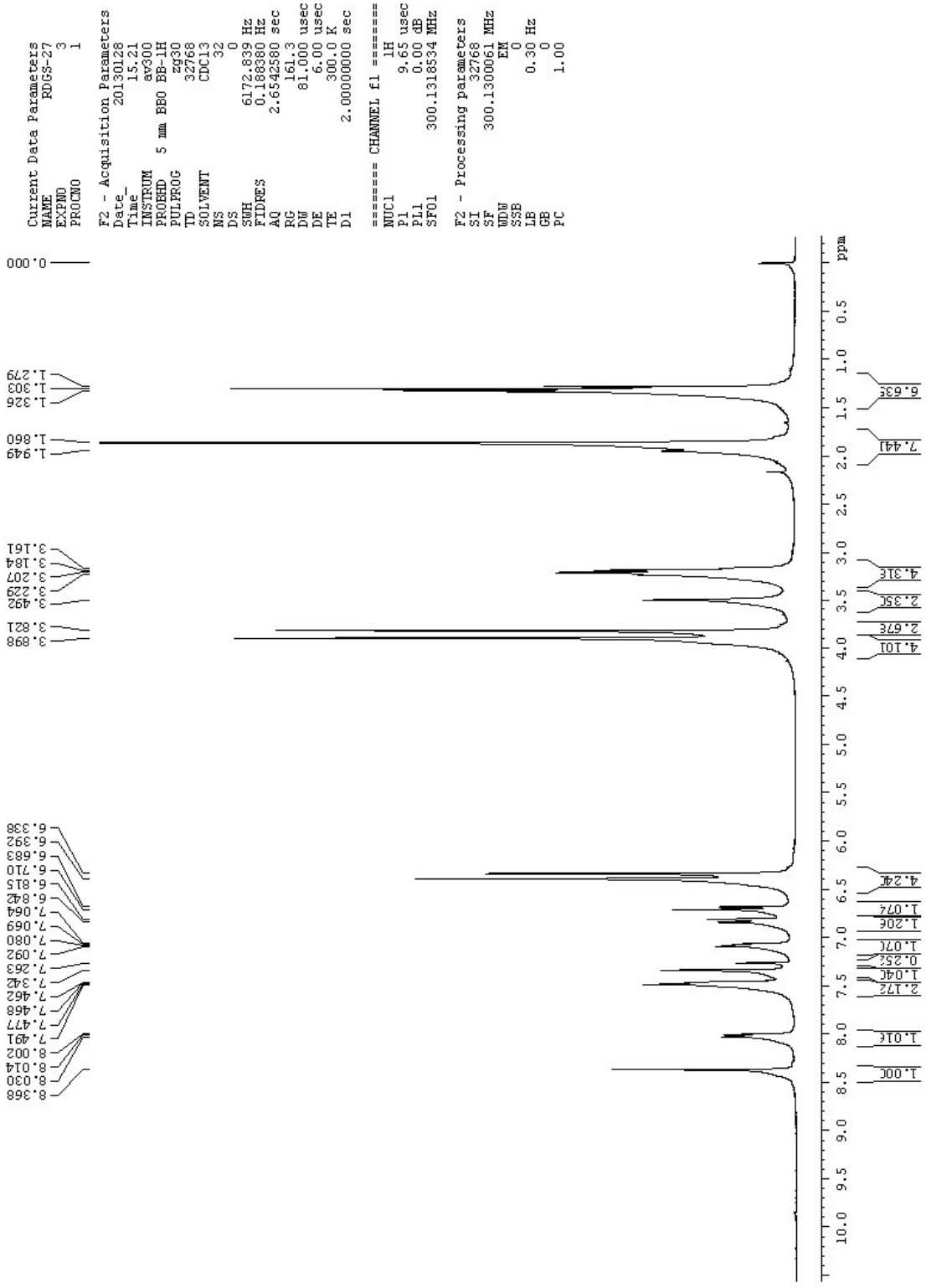

Figure S1: ^{1}H NMR spectrum of RDP-1.

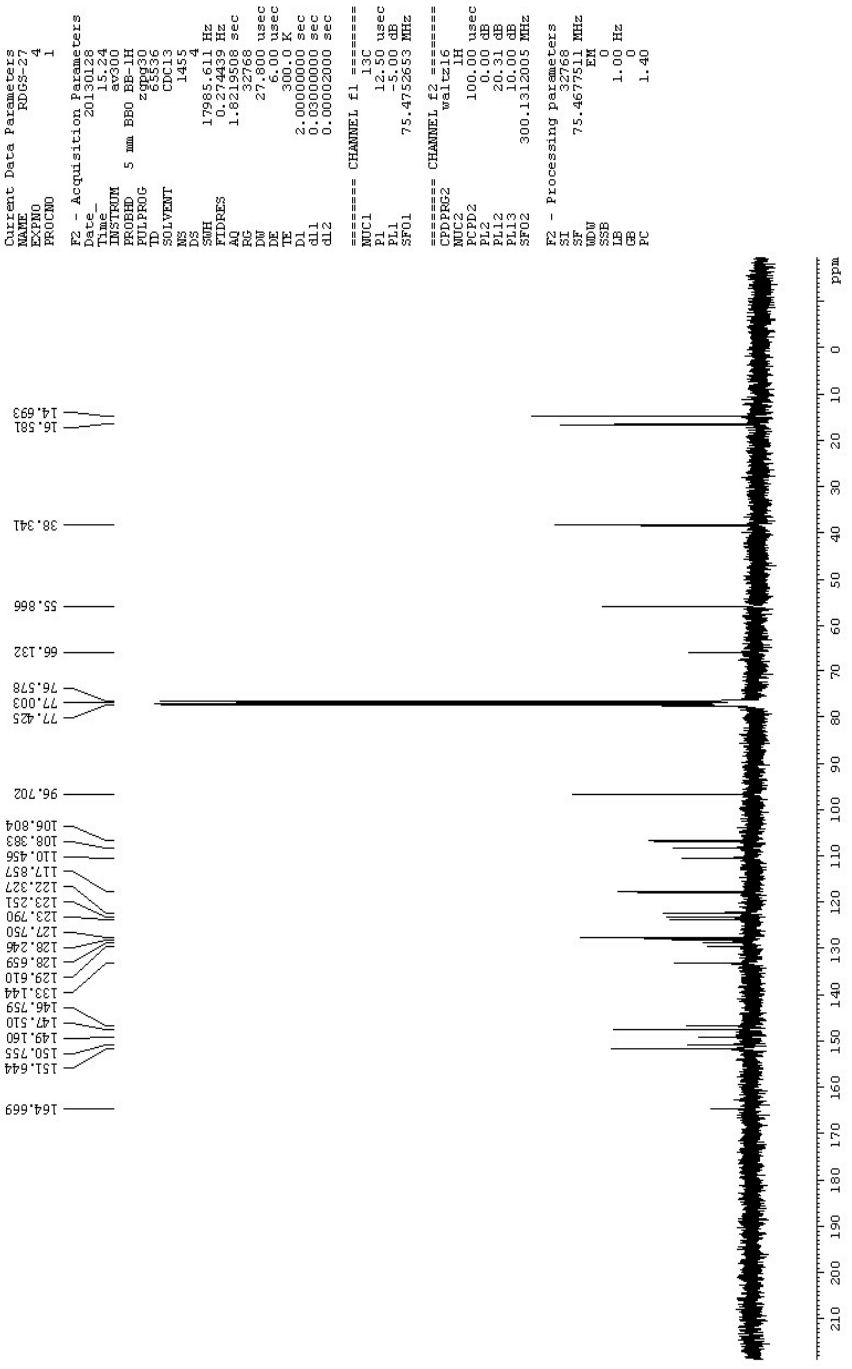

Figure S2: C^{13} NMR spectrum of RDP-1.

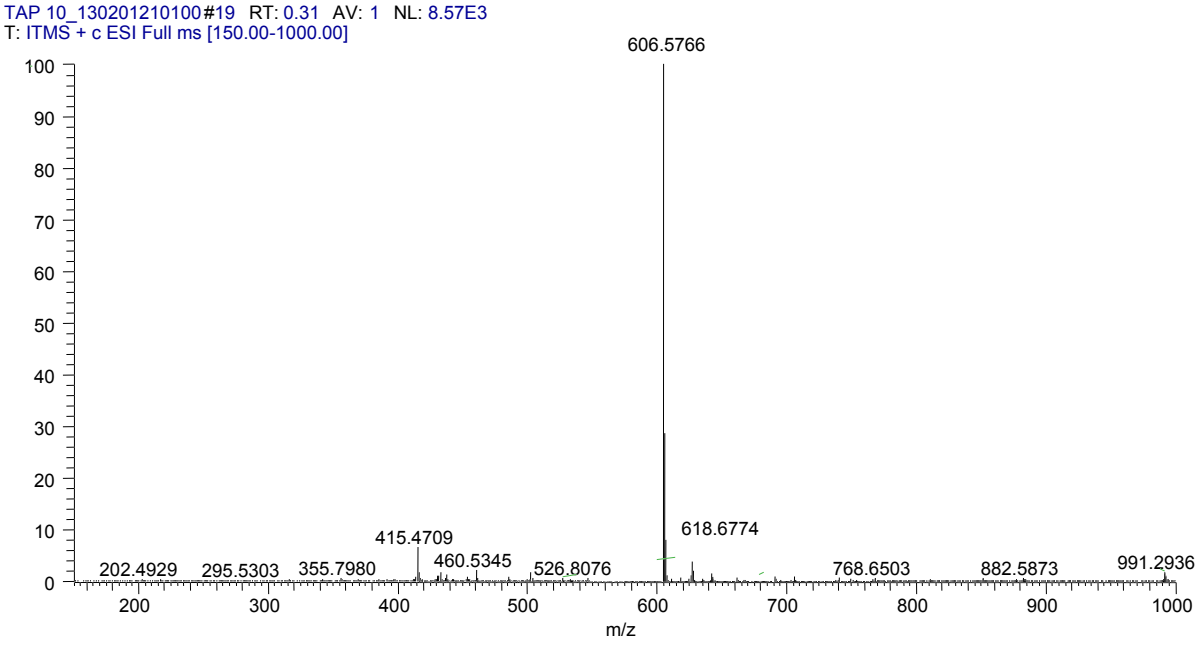

Figure S3: ESI-MS spectrum of RDP-1

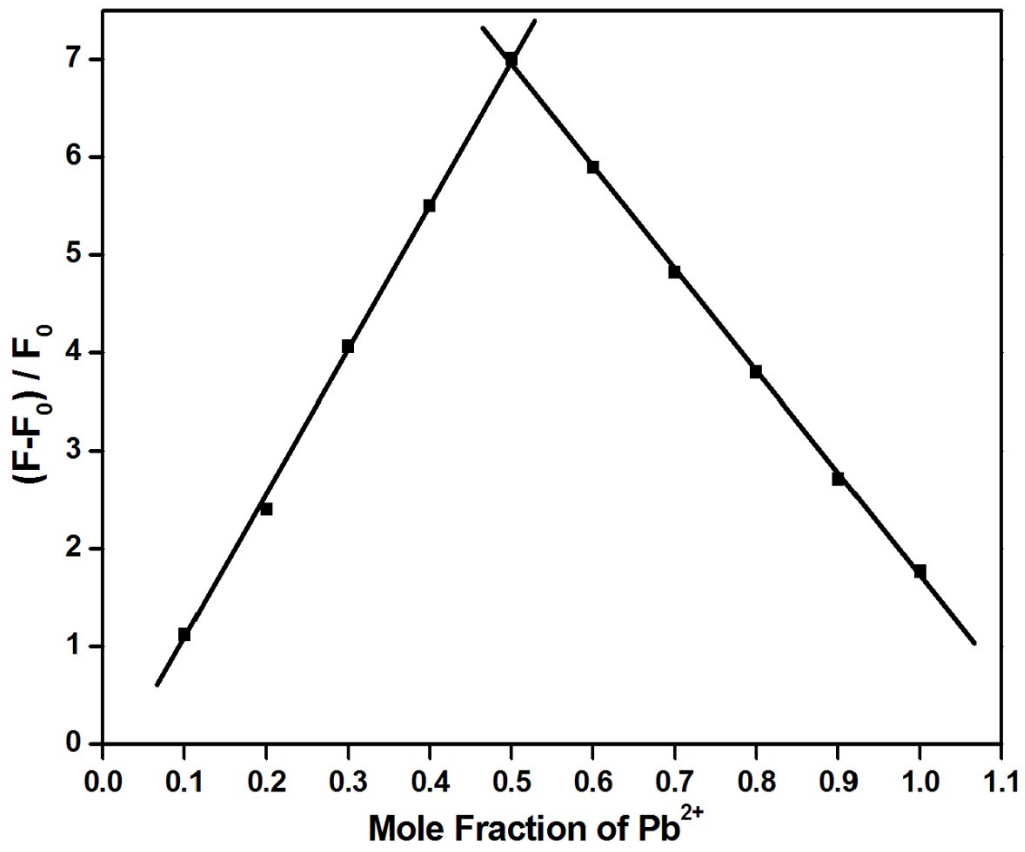

Figure S4: The Job's plot of mole fraction of Pb^{2+} *vs* fluorescence intensity at λemission = 550 nm.

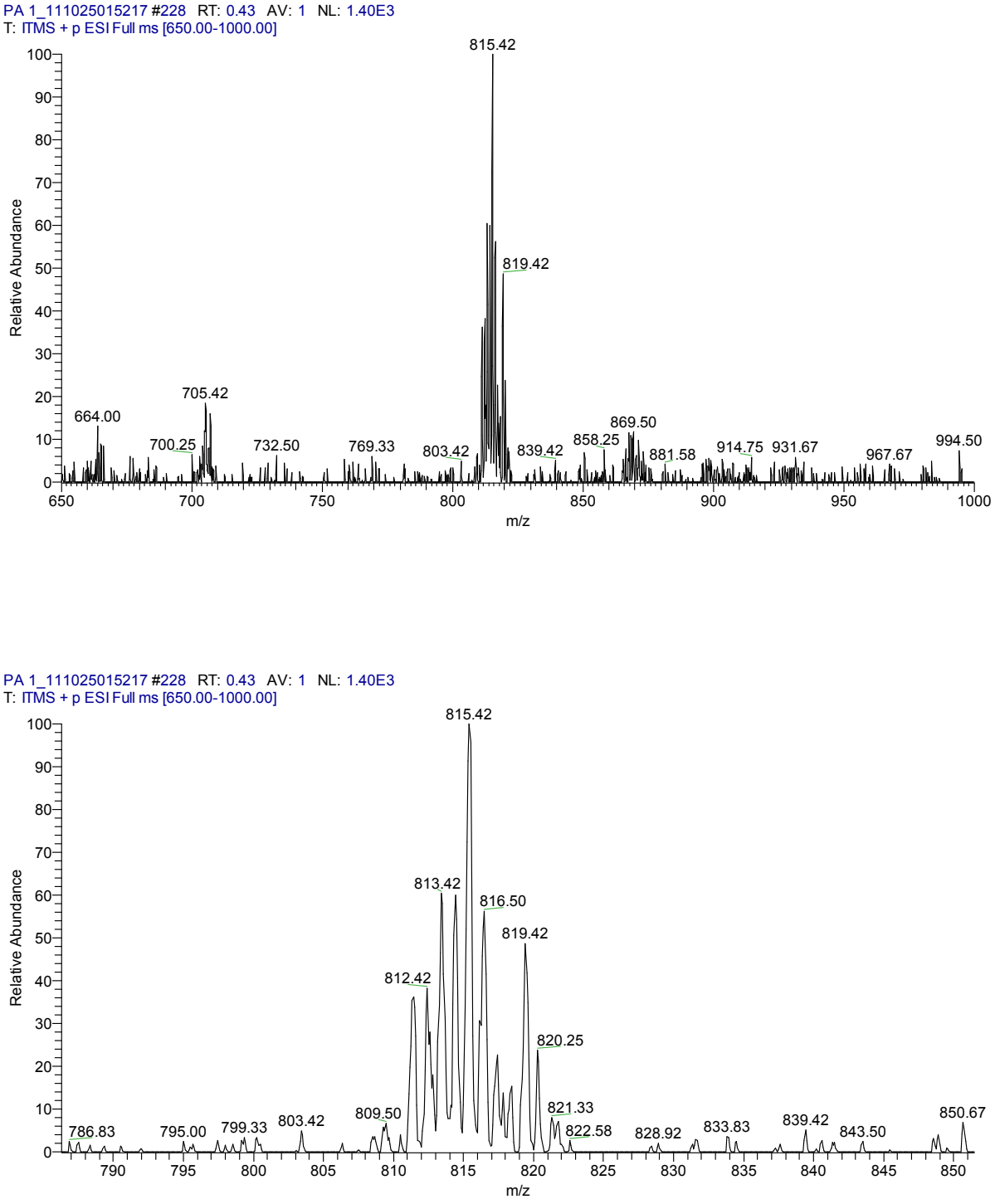

Figure S5: ESI-MS spectrum of RDP-1 + Pb^{2+} (Normal and Expanded view)

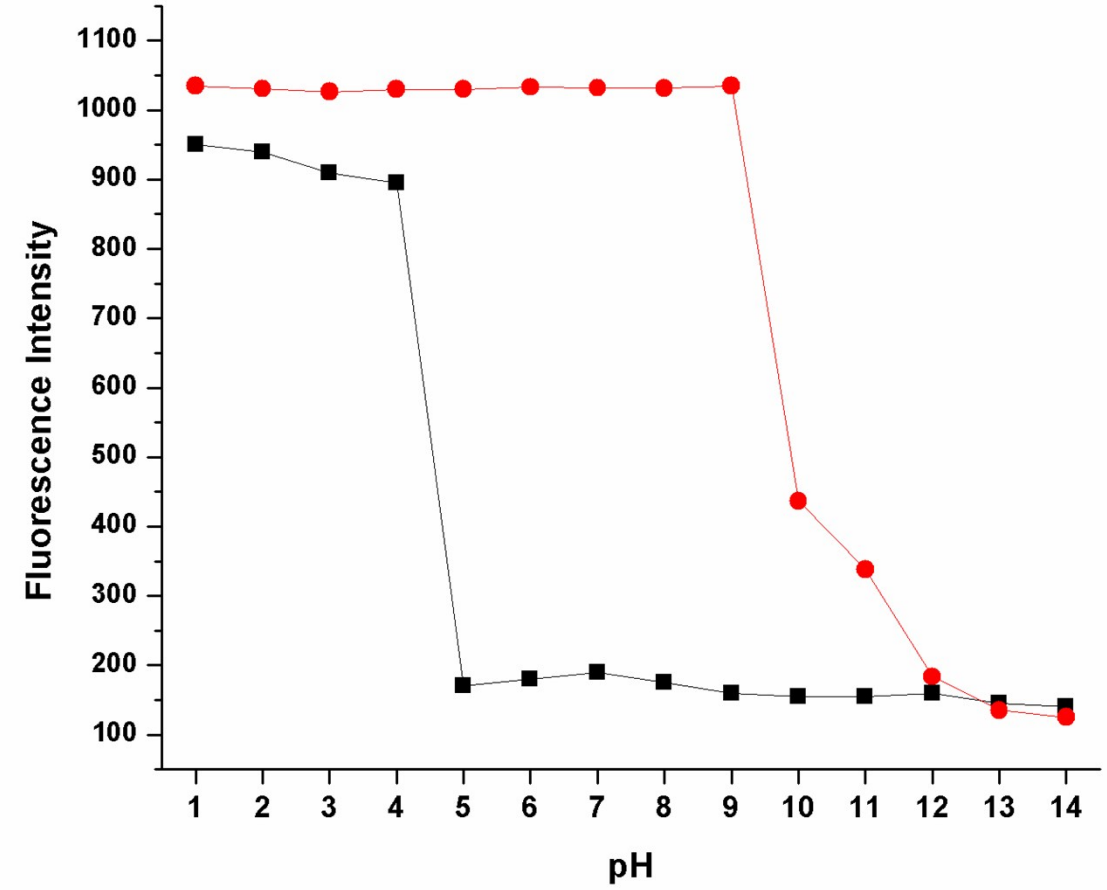

Figure S6: Effect of pH on the fluorescence of RDP-1(black) and RDP-1 with Pb^{2+} (red).

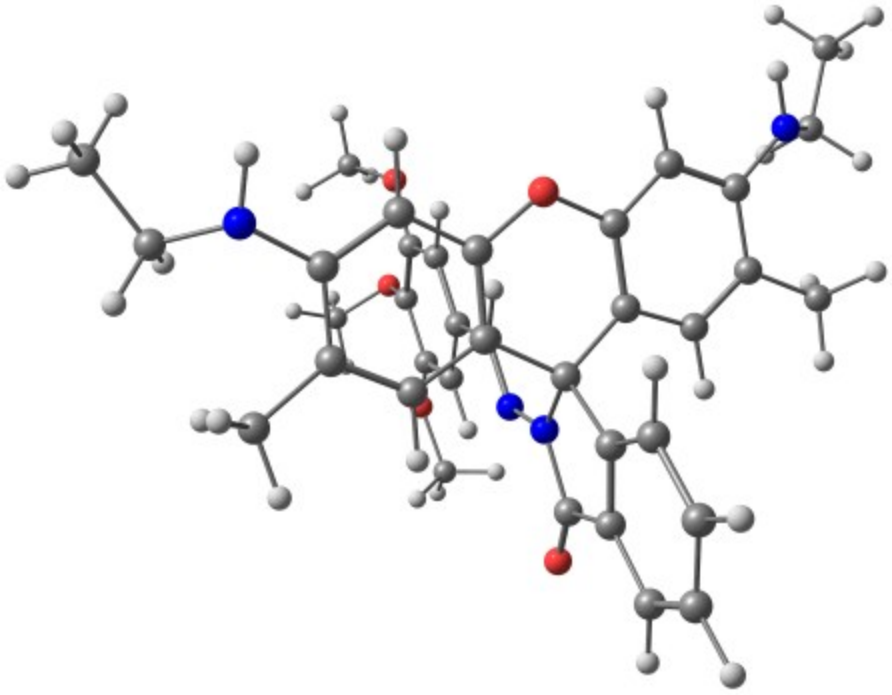

Figure S7: optimized geometry of the probe RDP-1

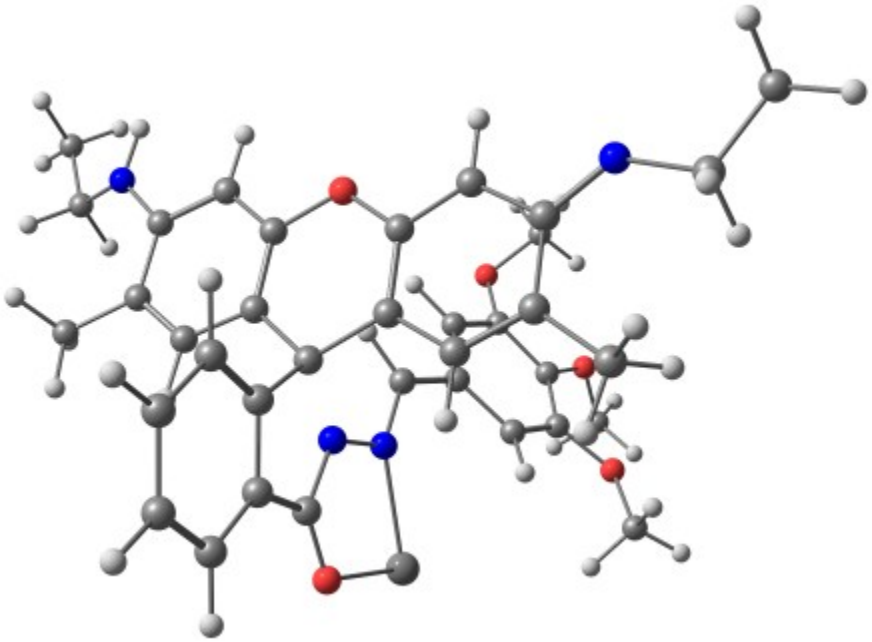

Figure S8: optimized geometry of the Pb^{2+} complex of probe RDP-1

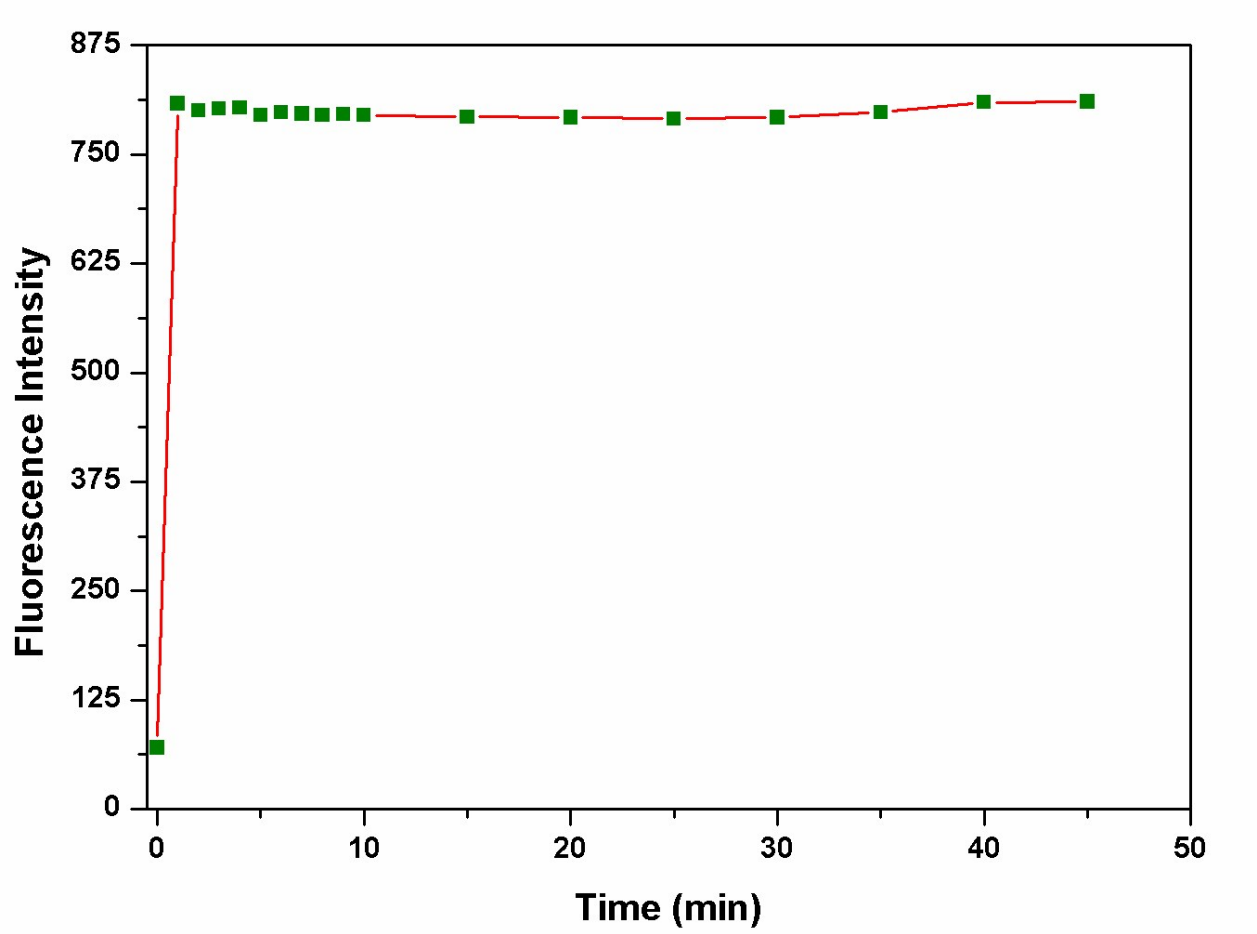

Figure S9: Plot of fluorecence intensity vs RDP-1+Pb^{2+} in different time intervals.

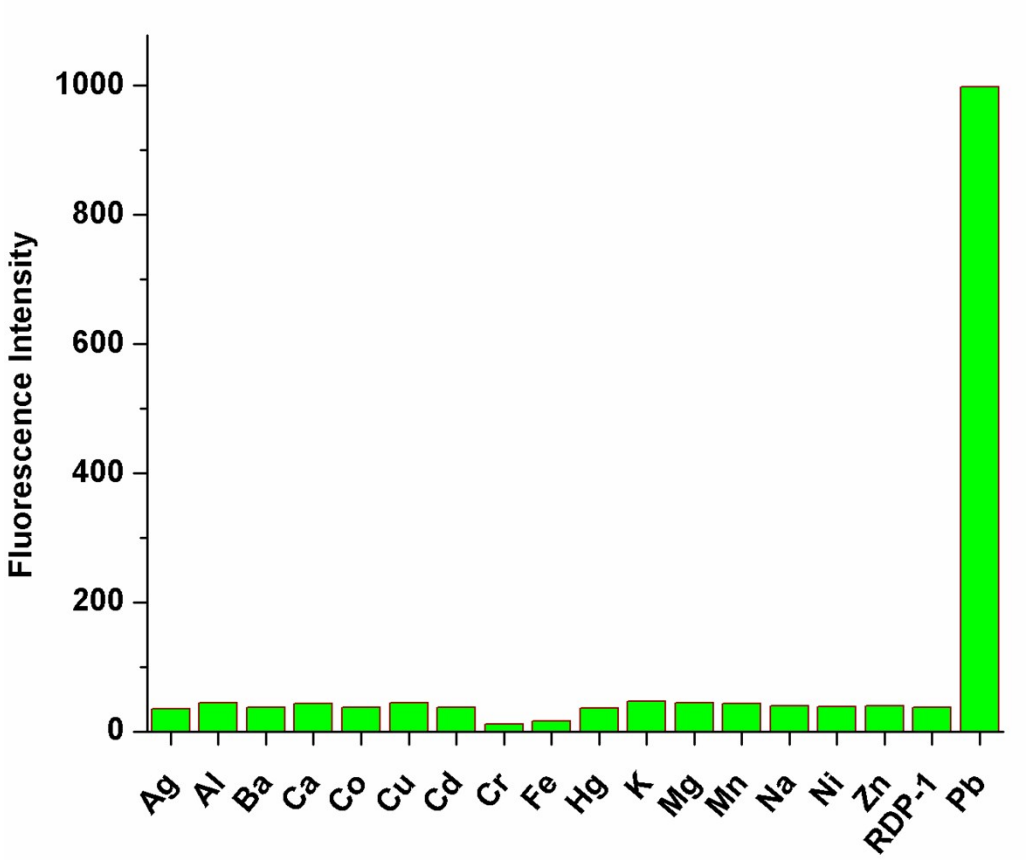

Figure S10: Fluorescence response of probe RDP-1(10μM) towards various metal ions (10 μM).

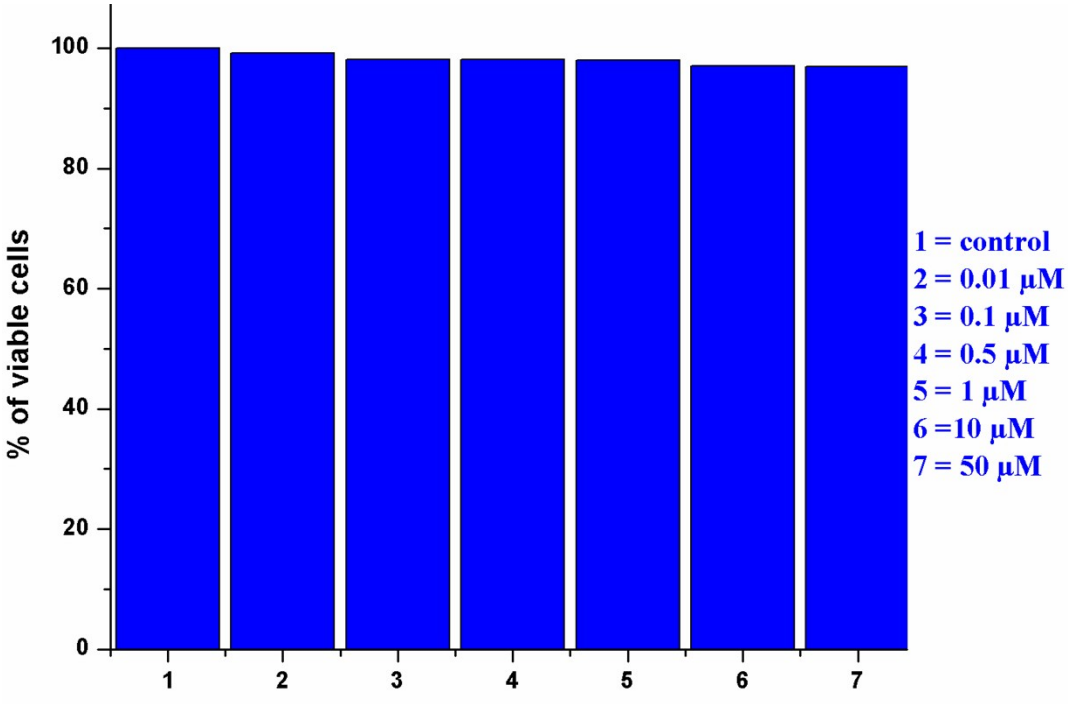

Figure S11: Plot of % of viable cells with conc of Probe RDP-1, MTT assay of HeLa cells treated with different concentrations of **RDP-1.**