# Pyrophosphate sensing by a fluorescent $Zn^{2+}$ bound triazole linked imino-thiophenyl conjugate of calix[4]arene in HEPES buffer medium: Spectroscopy, microscopy and cellular studies

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## Contents

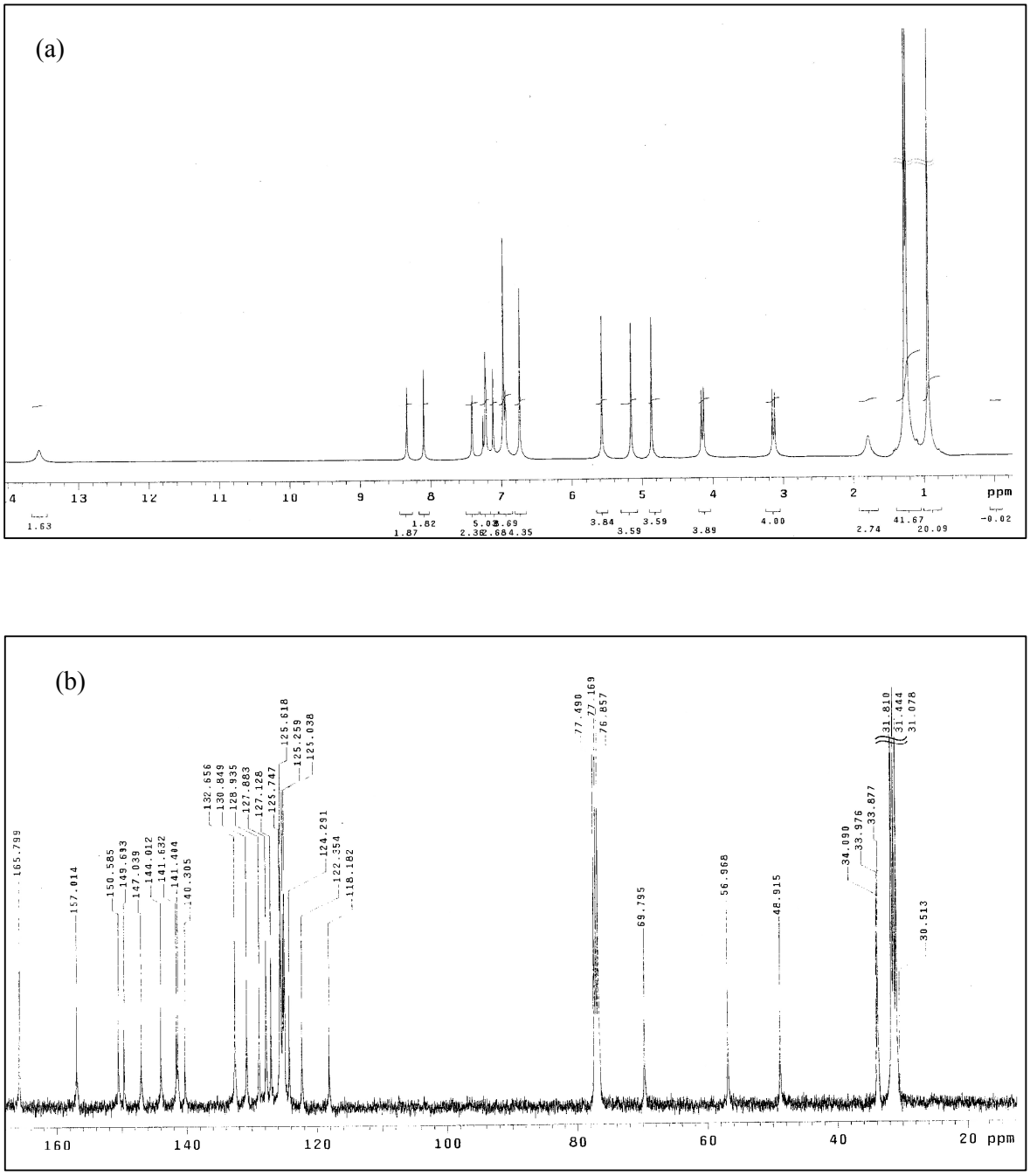
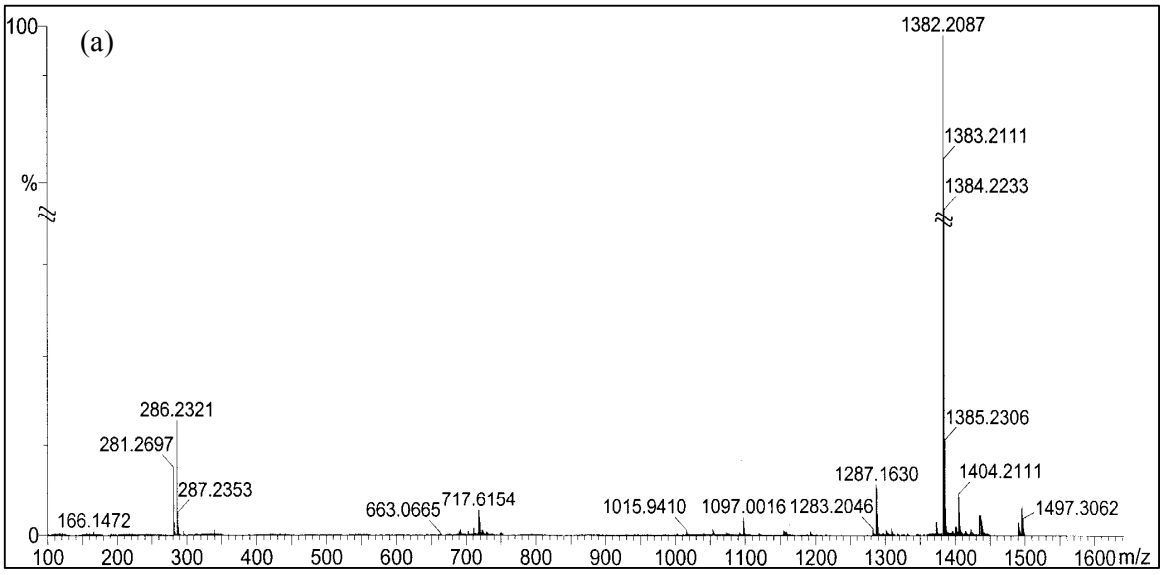



**Figure S1**: Spectra for **L**: (a) $^{1}$H NMR; (b) $^{13}$C NMR.




(b)

**Elemental Composition Report** **Page 1**

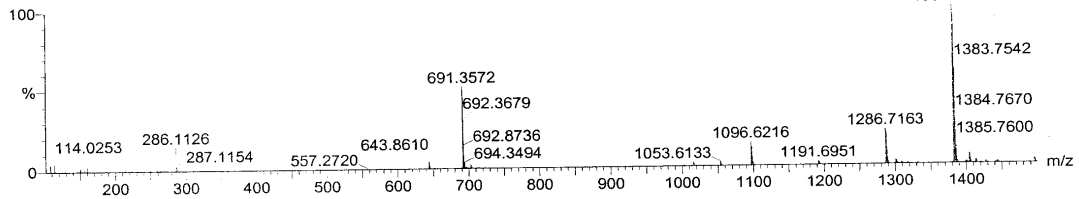
**Single Mass Analysis (displaying only valid results)**
Tolerance = 200.0 mDa / DBE: min = -1.5, max = 50.0
Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron Ions
156 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)

Micromass : Q-Tof micro (YA-105) Dept. Of Chemistry I.I.T.(B) 08-Apr-201118:06:28

C84H100N8O6S2
CPR-RKP-OC1TMA 24 (0.240) AM (Top,5, Ar,5000.0,556.28,1.00); Sm (Md, 6.00); Sb (5,40.00 ); Cm (1:39)

TOF MS ES+
1381.7302 1.35e5




| | | | | | | |
|---|---|---|---|---|---|---|
| Minimum: | | | | -1.5 | | |
| Maximum: | | 200.0 | 15.0 | 50.0 | | |
| Mass | Calc. Mass | mDa | PPM | DBE | Score | Formula |
| 1381.7302 | 1381.7286 | 1.7 | 1.2 | 38.5 | 1 | C84 H101 N8 O6 S2 |

**Figure S2**: Spectra for **L**: (a) ESI-MS; and (b) HRMS.

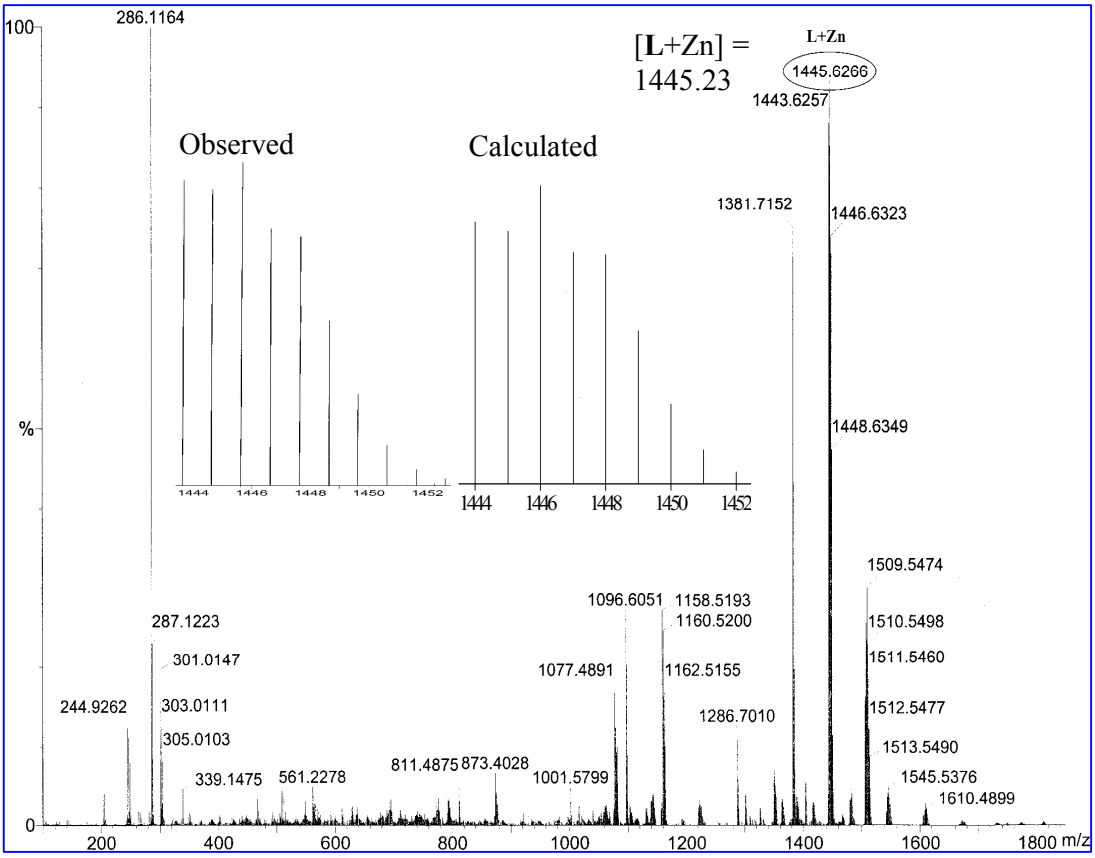



**Figure S3**. ESI MS spectrum of the *in situ* prepared [Zn**L**] complex of **L** and the isotopic peak pattern (observed & calculated matching) supports the presence of $Zn^{2+}$.

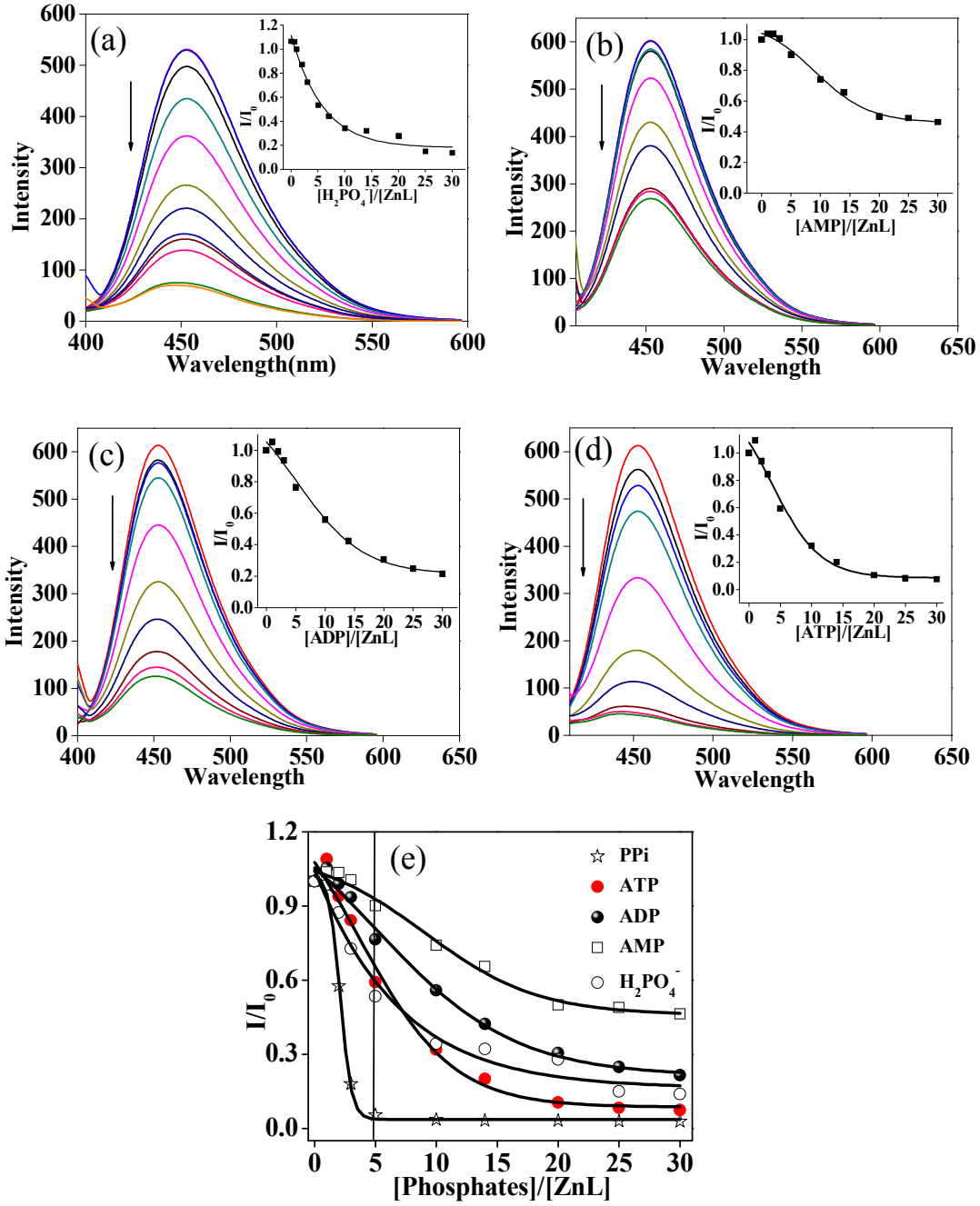



**Figure S4**: Fluorescence spectra obtained for the titration of the [Zn**L**] with phosphates based anion and nucleotides in aqueous ethanolic HEPES buffer solution (1:2) at pH = 7.4, $\lambda_{ex}$ = 454 nm. [Zn**L**] = 10 μM.

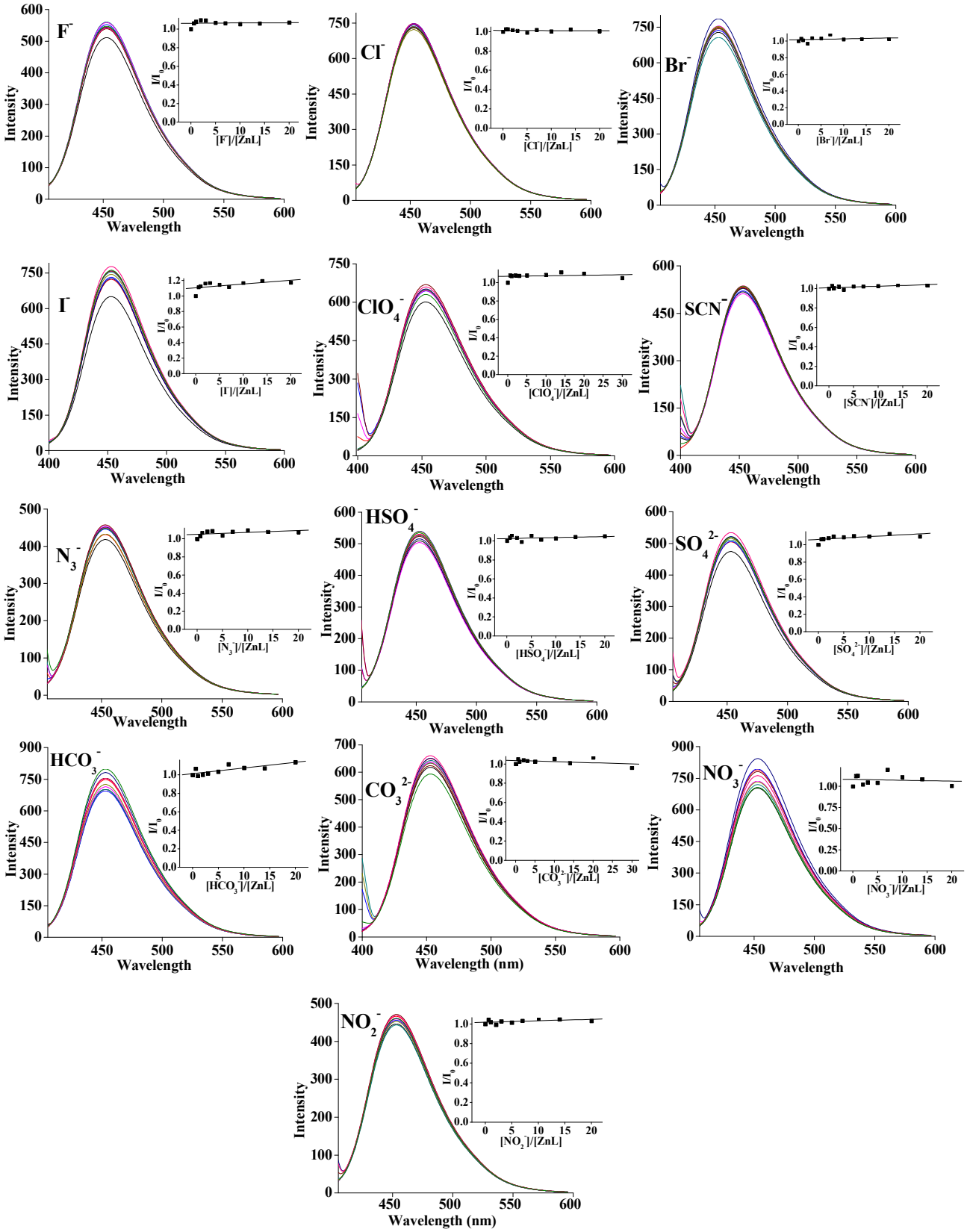



**Figure S5**. Fluorescence spectra obtained for the titration of [Zn**L**] with different anions in aqueous ethanolic HEPES buffer solution (1:2) at pH = 7.4, $\lambda_{ex}$ = 454 nm. [Zn**L**] = 10 μM..

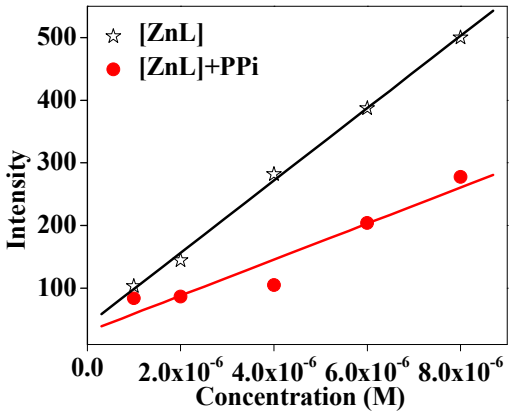



**Figure S6**: Fluorescence of [Zn**L**] and {[ZnL] + PPi} in aqueous ethanolic HEPES buffer solution (pH=7.4) by keeping [Zn**L**] to PPi ratio as 1:2. This is used for obtaining the minimum detection of limit of PPi by [Zn**L**].

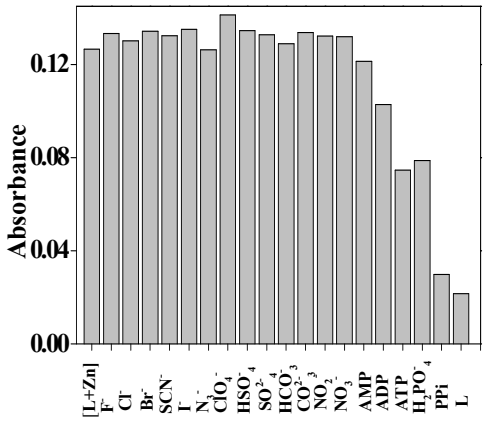



**Figure S7**: Histogram showing the absorbance at 380 nm band in the titration [Zn**L**] with anions in *aqueous buffer solution*.

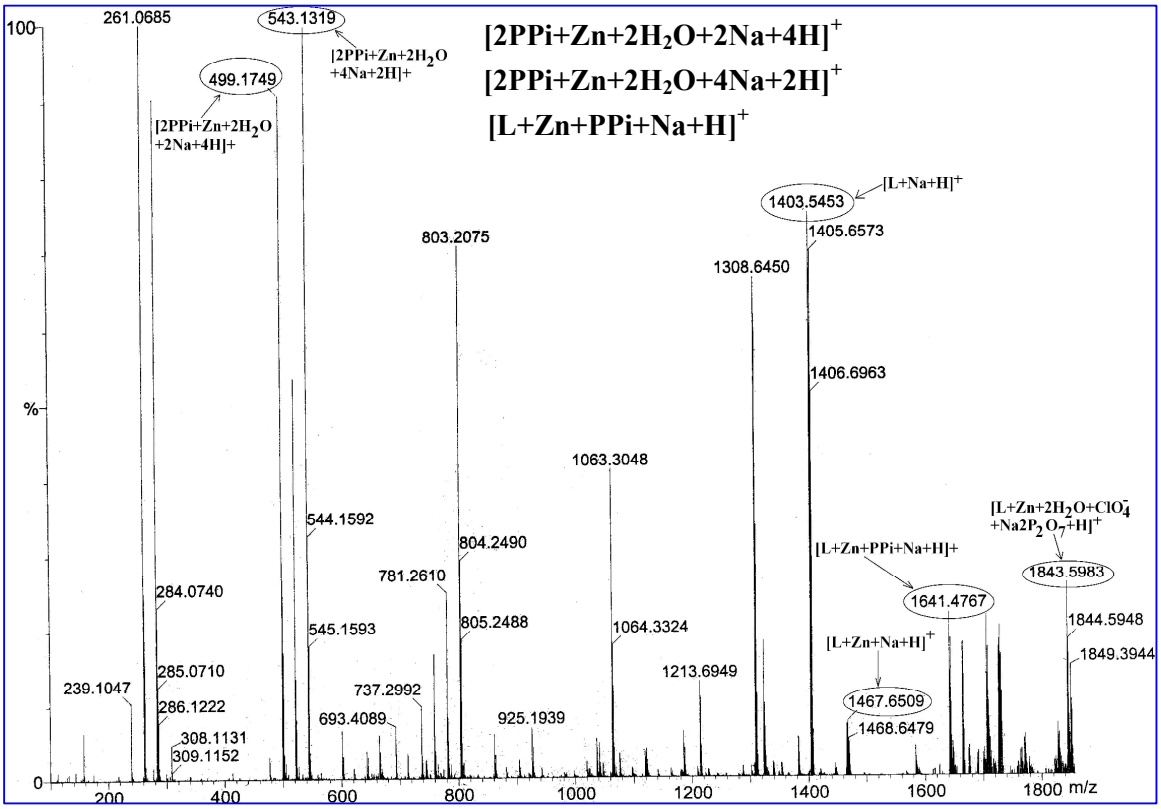



**Figure S8**: ESI MS spectrum obtained during the titration of [Zn**L**] with PPi and proposed species based on ESI Mass.

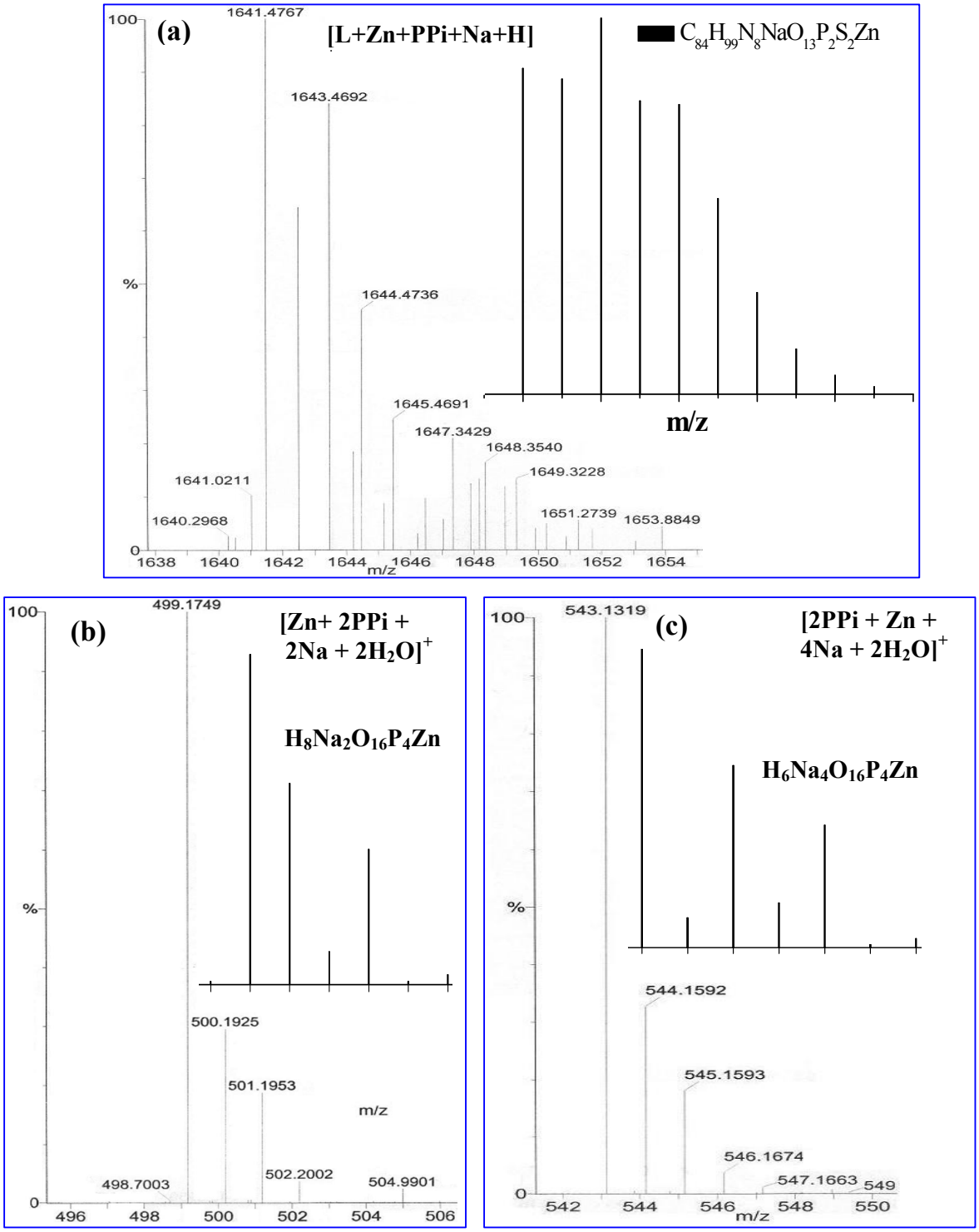



**Figure S9**: Isotopic peak pattern (observed and calculated) obtained from the titration of [Zn**L**] with PPi: (a) for 1641 corresponds to [**L**+Zn+PPi+Na+H]$^+$ species; (b) for 499 corresponds to [Zn+ 2PPi + 2Na + 2$H_2O$]$^+$ species; (c) for 543 corresponds to [2PPi + Zn + 4Na + 2$H_2O$]$^+$ species.

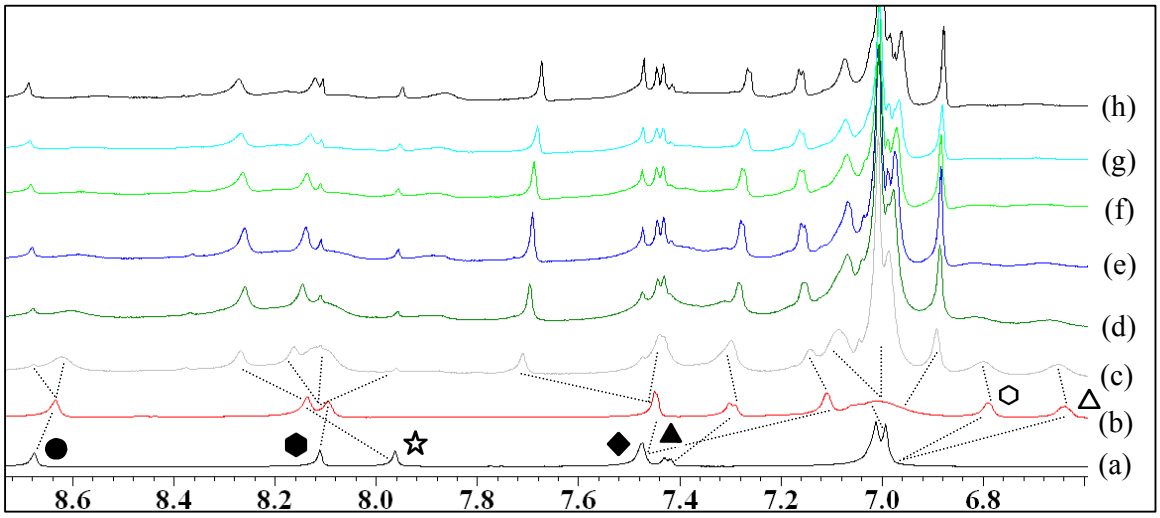



**Figure S10**: $^{1}$H NMR spectra for the titration of [Zn**L**] with PPi (experimental section) in DMOS-$d_6$: (a) **L**; (b) **L**+ 2 equiv. of $Zn^{2+}$ and thereafter [Zn**L**] + 'n' equivalents PPi: (c) n = 0.5; (d) n = 1.0; (e) n = 2.0; (f) n = 3.0; (g) n = 4.0; (h) n = 5.0. ● = imine-H; ⬢ = Triazole-H; ☆ = Calix-OH; ⬡,▲, △ = thiophene-H.

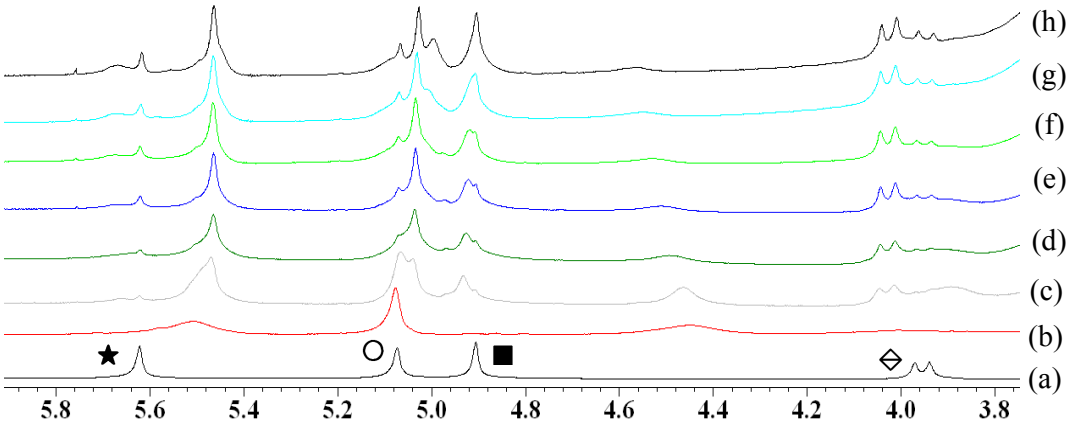



**Figure S11**: $^{1}$H NMR spectra for the aliphatic region of [Zn**L**] when titrated with PPi in DMSO-$d_6$; (a) **L**; (b) **L**+ 2 equiv. of $Zn^{2+}$ and thereafter [Zn**L**] + 'n' equivalents PPi: (c) n = 0.5; (d) n = 1.0; (e) n = 2.0; (f) n = 3.0; (g) n = 4.0; (h) n = 5.0; ★ = $CH_2$– connecting to triazole with aldimine; ○ = $CH_2$– connecting to calix[4]arene with triazole; ■ = = Thiophene-$CH_2$; ⋄ = calix[4]arene bridged-$CH_2$.

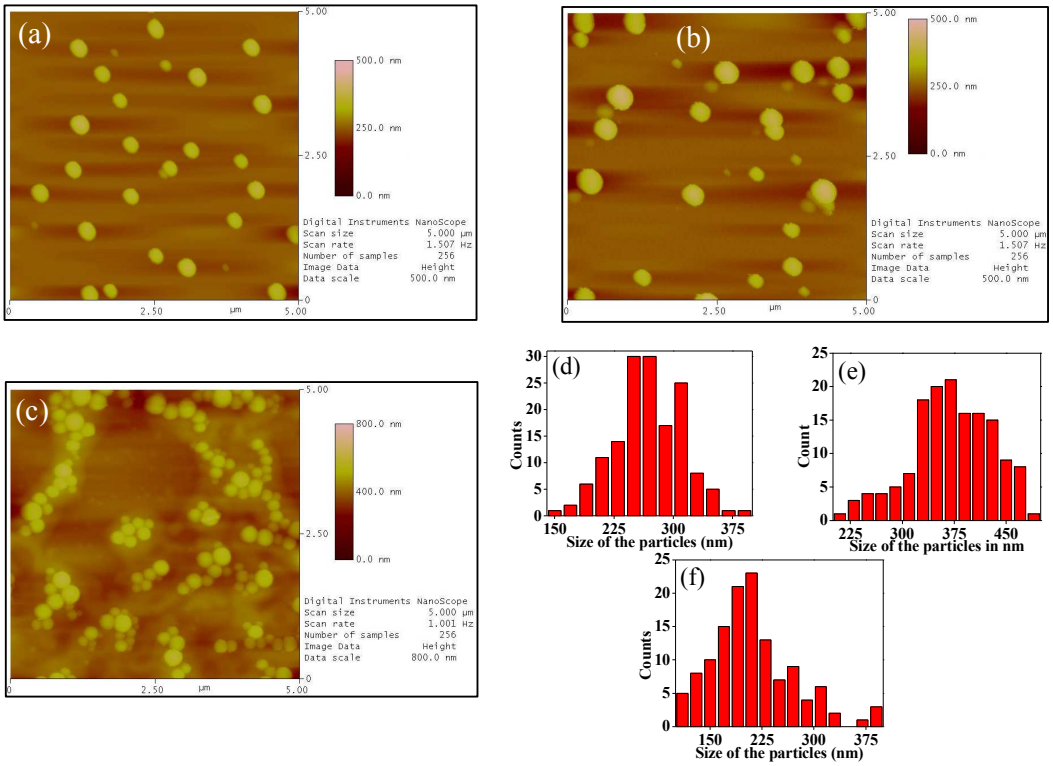



**Figure S12**: AFM micrographs: (a) for **L**; (b) for [**L**+$Zn^{2+}$]; (c) for {[**L**+$Zn^{2+}$] + PPi}. Particle distribution plots: (d) for **L**; (e) for [**L**+$Zn^{2+}$]; (f) for {[**L**+$Zn^{2+}$] + PPi}.

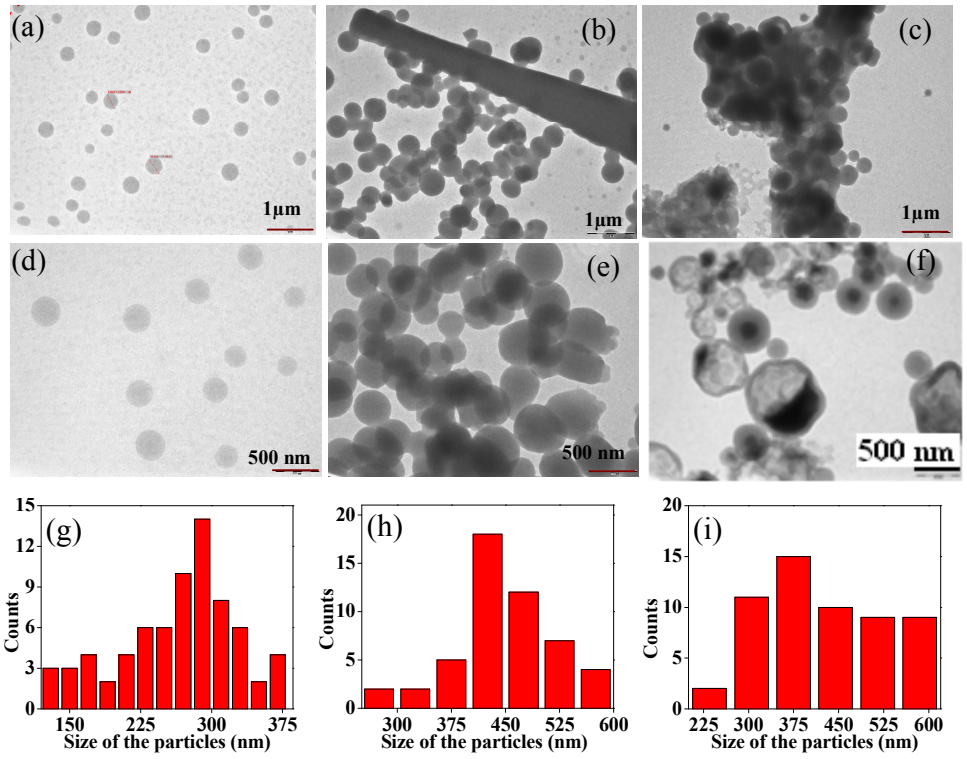



**Figure S13**: TEM micrographs: (a, d) for **L**; (b, e) for [**L**+$Zn^{2+}$]; (c, f) for {[**L**+$Zn^{2+}$] + PPi}. Particle distribution plots: (g) for **L**; (h) for [**L**+$Zn^{2+}$]; (i) for {[**L**+$Zn^{2+}$] + PPi}.

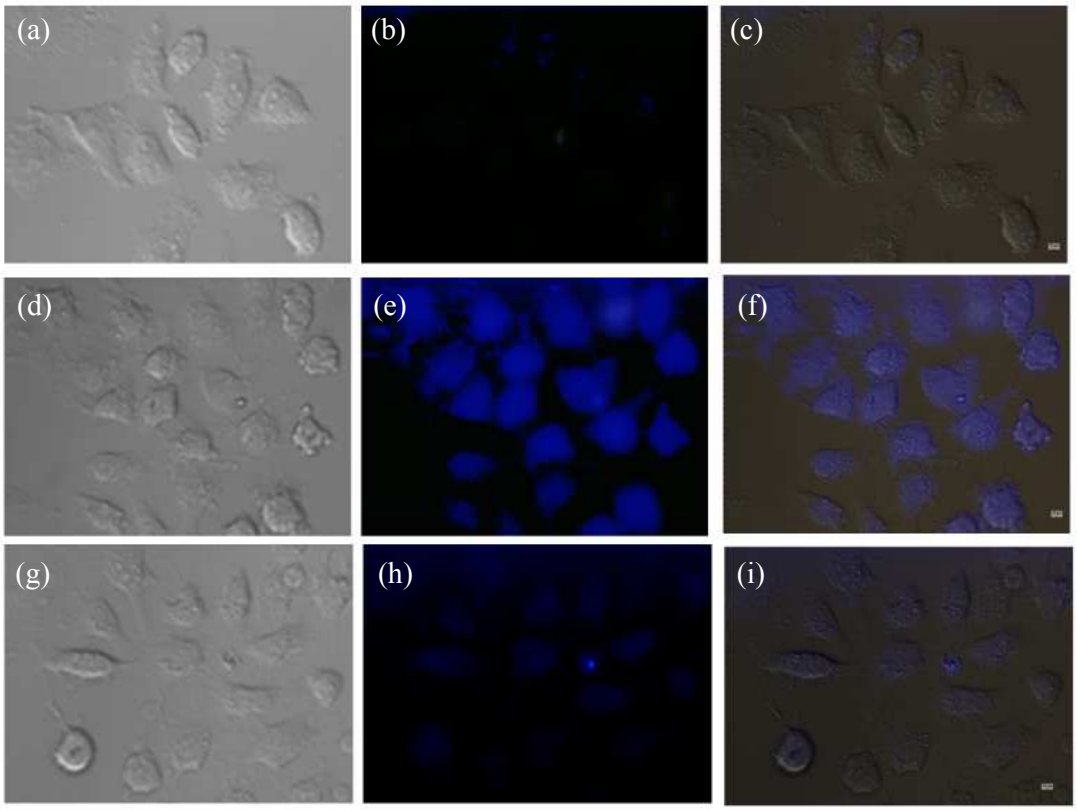



**Figure S14**: Another set of fluorescence images obtained from the HeLa cells (excitation at ~358 nm and emission at ~ 461 nm) upon treatment in PBS buffer at pH = 7.4: (a) DIC image when treated with **L** (10 μM); (b) fluorescence image of that given under (a); (c) a merge image of (a) and (b). (d) DIC microscopy image of the HeLa cells treated with **L** followed by 20 μM of $Zn^{2+}$/Pyrithione (1:1) solution; (e) fluorescence image of that given under (d); (f) a merge image of (d) and (e). (g) DIC microscopy image of the HeLa cells treated with [**L**+$Zn^{2+}$**]** followed by 50 μM of pyrophosphate; (h) fluorescence image of that given under (g); (i) a merge image of (g) and (h). Scale bar is 10 μm.

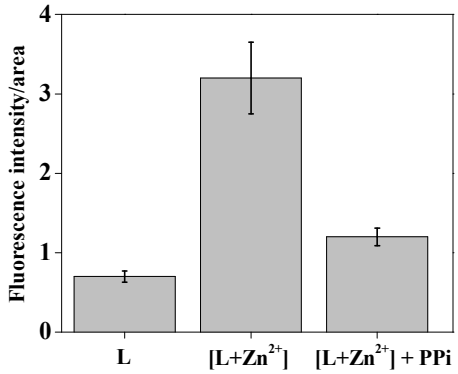



**Figure S15**: Histogram showing the fluorescence changes of **L** in the HeLa cells incubated with $Zn^{2+}$ and followed by PPi as measured by selecting the area of the cells.

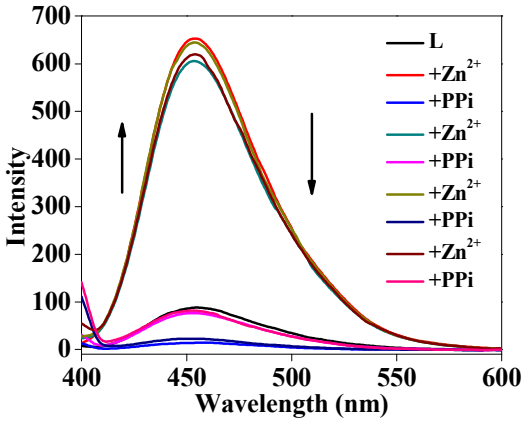



**Figure S16**: Fluorescence titration shows the reversibility of the receptor for sensing $Zn^{2+}$ and PPi in aqueous ethanolic (1:2 *v/v*) HEPES buffer (pH = 7.4), [**L**] = 10 μM; $\lambda_{ex}$ = 390 nm.