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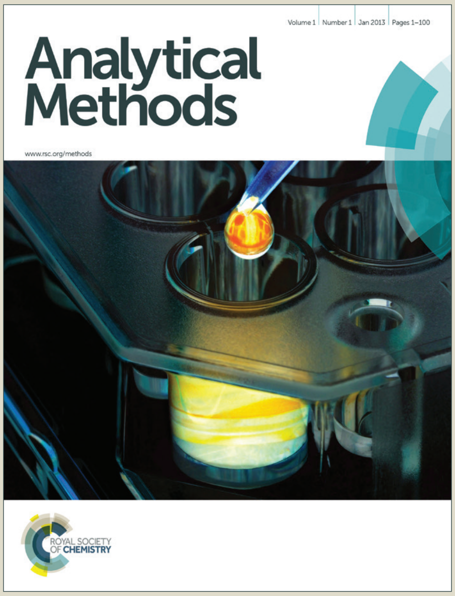

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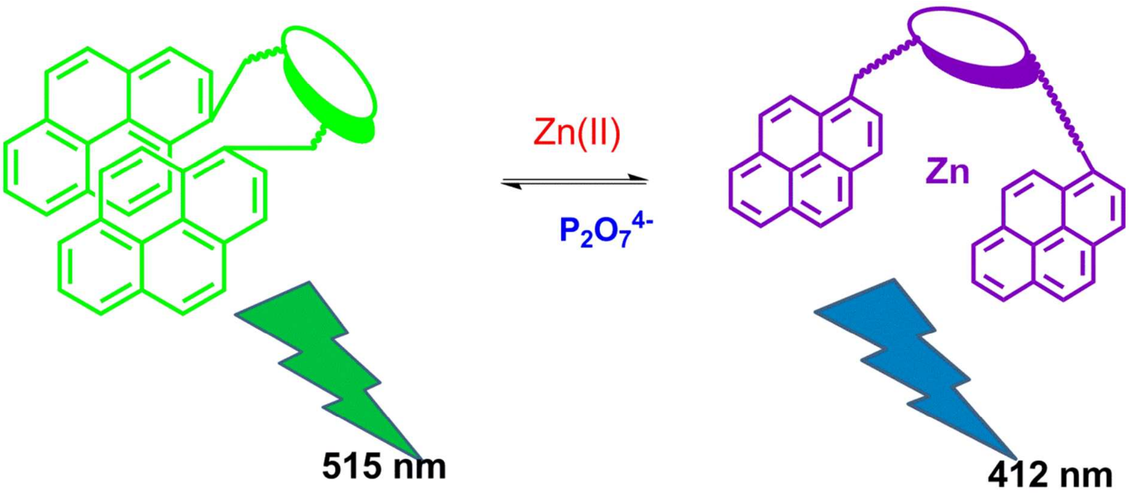
Zn(II)
P2O7 4-
Zn
515 nm
412 nm

Cite this: DOI: 10.1039/c0xx00000x

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ARTICLE TYPE

Pyrene based Selective - Ratiometric Fluorescent Sensing of Zinc and Pyrophosphate ions.

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Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX

DOI: 10.1039/b000000x

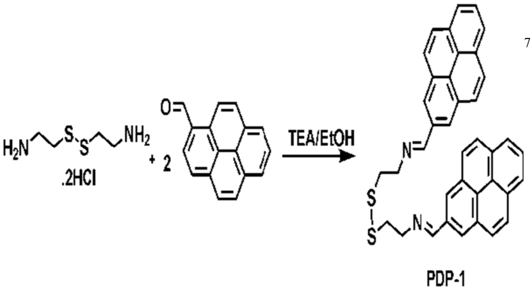
A simple pyrene-based receptor has been synthesized. It shows a colorimetric and ratiometric fluorescent detection of zinc in aqueous solution. The ratiometric fluorescent change has been accounted to the conformational change of the probe. The ratiometric fluorescence change has been further supported by DFT/TDDFT calculations. PDP-1+ Zn^{2+} complex is also further successfully utilized for ratiometric fluorescence detection of pyrophosphate anions in buffered aqueous solution. The applicability of these probes for real sample analysis for the detection of Zn(II) and pyrophosphate ions has been also studied.

Introduction:

Zinc ion (Zn^{2+}) is the second most abundant transition metal ion in the human body after iron. Many studies reveal that Zn^{2+} is involved in a number of biological processes, such as the brain function and pathology, gene transcription, immune function, and mammalian reproduction.[1] Increased levels of zinc are known to play a key role in potential neurological disorders such as Alzheimer's and Parkinson's disease[2] however a detailed understanding of the role played by the Zn(II) ion in cell homeostasis, signal transduction, and translocation still remains a challenge. Within the past decade, pioneering research in PET,[3] ICT,[4] FRET[5] based fluorescent chemosensors have made significant progress in understanding the bioinorganic chemistry and coordination properties of Zn(II). The development of highly selective and ratiometric fluorescent chemosensors for Zn^{2+} ions is still an important task,[6-9] although some fluorescent sensors for Zn^{2+} by the ratio of the emission intensity changes at two wavelengths have been reported in the literature [10-13]. For the construction of efficient optical chemosensors, fluorescence is particularly attractive. pyrene subunits are widely employed due to their well-known photophysical properties as well as their characteristic and environment-sensitive monomer or excimer emissions. Particularly, the introduction of two pyrene moieties can be situated closely enough to yield excimer emission. Upon coordination with a specific guest ion, the resulting compound could be fine-tuned to yield monomer and/or excimer emissions depending on the orientation of the two pyrene moieties.[14-18] Furthermore, only a few ratiometric fluorescent probes with pyrene for Zn^{2+} have been found in the literature involves multistep synthesis.[19-21] Phosphates act as substrates or inhibitors by reversibly coordinating to Zn(II) ions in the enzymes. Such compounds were initially designed as small molecule models of enzyme active sites.[22] It plays numerous important roles in cell biology[22]. ATP is also called as energy currency of the living cells[23]. Phosphates are involving in the modulation of the ion channels[24] apart from these it also involves in the DNA replication and transcription[25]. The enhanced binding ability of phosphates with Zinc makes that the utilization of a zinc ion complex as a binding site for phosphates has become the most popular approach.[23,24] Due to the simplicity and high sensitivity of fluorescence methods when compare to other methods are predominantly attractive for the detection of phosphate ions [26]. Even though, only few number of reports of an effective ratiometric fluorescent chemosensor for phosphate ions[27]. Herein we report simple, convenient, rapid single step synthesis of the probe which shows selective fluorescent switching behaviour towards Zinc ions ratiometrically by the excimer/monomer emission of pyrene moiety. During addition of phosphate anions PDP-1+ Zn^{2+} complex showed a ratiometric fluorescence change in buffered aqueous solution.

Results and Discussion:

PDP-1 was synthesized by a single step reaction between 1-pyrene carboxaldehyde and Cystamine dihydrochloride in the presence of triethylamine (Scheme-1). PDP-1 was characterized by NMR and Mass spectral techniques (Figure S1, S2 and S3 in ESI).



Scheme-1: synthesis of PDP-1

Spectroscopic measurements were carried out in aqueous HEPES buffer (10 mm HEPES pH 7.4) containing 70% CH_3CN The absorption spectrum of PDP-1 displays characteristic spectral features of pyrene, with absorption bands at 278, 332 and 348 nm. Upon addition of Zn^{2+} ions to the PDP-1 there was significant change in the absorption bands around 390–400 nm (Figure 1). This is also responsible for the perceptible to deepening of color from pale yellow to darkening. This is directly related to an increase in the molecular weight of chromogens that is due to the formation of PDP-1+Zn^{2+} complex. The absorption spectra with several metal cations (Fe^{3+}, Fe^{2+}, Ag^{+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Hg^{2+}, K^{+}, Mg^{2+}, Mn^{2+}, Na^{+}, Ni^{2+}, and Pb^{2+}) did not produce any appreciable change in UV-vis spectra[28] (Figure-1).

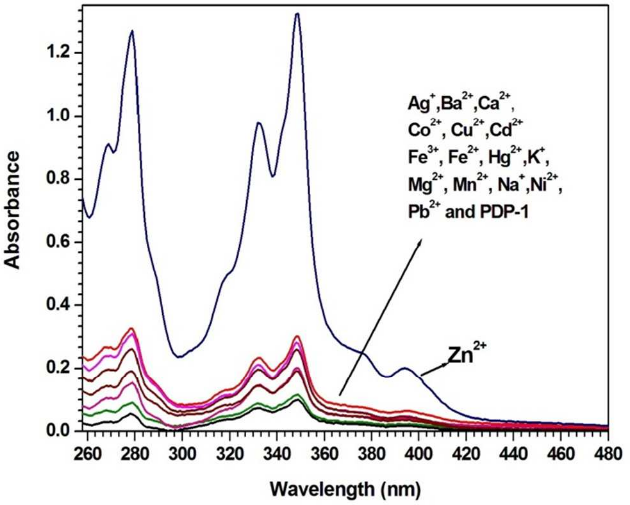


Figure-1: UV-vis absorption spectra of PDP-1 (10 µM) in the presence of Fe^{3+}, Fe^{2+}, Ag^{+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Hg^{2+}, K^{+}, Mg^{2+}, Mn^{2+}, Na^{+}, Ni^{2+}, $Pb^{2+}$and Zn^{2+} (10 µM) in aqueous HEPES buffer (10 mM HEPES, pH=7.4, H_2O: CH_3CN= 30:70).

The fluorescence spectra of PDP-1 (Figure 2) shows a strong excimer emission at 515 nm and a weak monomer emission at 390 and 412 nm (excitation wavelength 340 nm), with an intensity ratio of monomer to excimer emission ($I_M/I_E \approx I_{412}/I_{515}$) = 0.21. The formation of an excimer band at 515 nm indicates a strong face to face π–π stacking between the two pyrene units. The incremental addition of zinc ions leads to the complete disappearance of excimer band at 515 nm and the concomitant increase in the fluorescence intensity at 412 nm (figure 3). In PDP-1, the ratio of monomer to excimer emission is barely changed with change in the concentration, indicating that the excimer emission results from an intramolecular excimer but not from intermolecular interactions. The green fluorescence of PDP-1 is attributed to the stacked conformation of two pyrene units; whereas change in the fluorescence after addition of Zn^{2+} is due to the conformational change from stacked pyrene into the non-stacked ones. There is very weak change in an emission band at 475 nm which may be due to the possibility of Intermolecular excimer formation in HEPES buffered acetonitrile solution. Upon addition of Zn^{2+} to the PDP-1 solution, the emission intensities at around 374-400 nm increased significantly, but the peak at 515 nm blue shifted to 470nm (Figure. 2) and the blue shift is attributed to the rigidity of the pyrene conformers. To study the selectivity of PDP-1 for Zn^{2+}, its fluorescent properties in the presence of various cations were examined in in HEPES buffer. The biologically significant metal ions, such as Ag^{+}, Ba^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+}, Fe^{2+}, Hg^{2+}, K^{+}, Mg^{2+}, Na^{+}, Ni^{2+} and Pb^{2+}, did not produce any change in the fluorescence behaviour of PDP-1(figure-S6 in ESI). The interference of other metal ions (Ag^{+}, Ba^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+}, Fe^{2+}, Hg^{2+}, K^{+}, Mg^{2+}, Na^{+}, Ni^{2+} and Pb^{2+}) in the detection of Zn(II) was studied by competition experiments using mixed solutions containing equimolar solution of Zn(II) and the solution of PDP-1indicates that the metal ions does not interfere the detection of Zinc(II) ions (Figure-4). In order to understand the binding stoichiometry of Zn^{2+} with PDP-1, the plot was constructed using Job's continuous variation method by measuring the fluorescence intensity ratios at different mole fraction of Zn^{2+}.

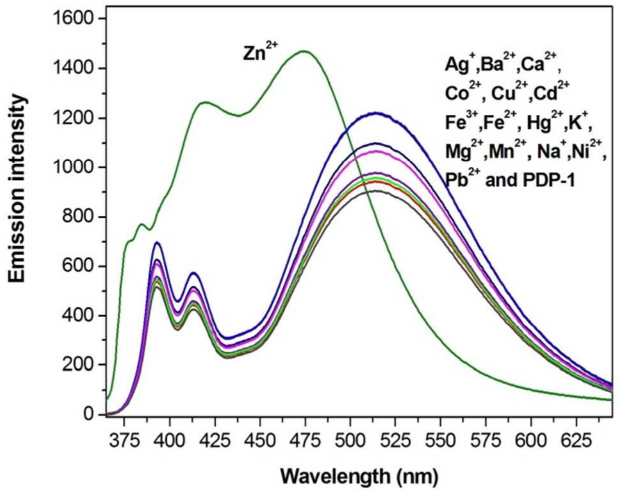


Figure-2: Fluorescence spectra of PDP-1 in the presence of Fe^{3+}, Fe^{2+} , Ag^{+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Hg^{2+}, K^{+}, Mg^{2+}, Mn^{2+}, Na^{+}, Ni^{2+}, $Pb^{2+}$and Zn^{2+} (10 µM) in aqueous HEPES buffer (10 mm HEPES pH 7.4) containing H_2O-CH_3CN (30:70). Excitation was performed at 350 nm.

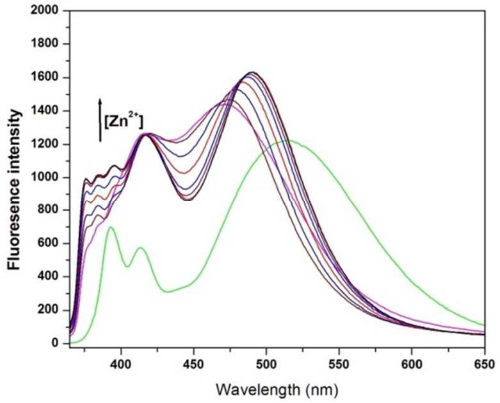


Figure-3: Fluorescence emission spectrum of PDP-1 (1 µM) upon addition of Zn^{2+} (0-1 equiv) in aqueous HEPES (10 mM HEPES, pH=7.4, H_2O: CH_3CN= 30:70).

It shows a maximum at 0.5 mol fraction of Zn^{2+} indicates the formation of a 1: 1 complex between PDP-1 and the Zn^{2+}ion (Figure-S7 in ESI). It was further supported by the peak at m/z 691.25 in ESI-MS spectrum corresponding to molecular weight of [PDP-1 + Zn^{2+} + MeOH+H_2O-H^{+}] (figure-S4 in ESI). There is a good linear relationship between ratio of excimer and monomer fluorescence intensity and the concentration of Zn^{2+}. The detection limit was found to be 8.548×10^{-8} M, (figure-S9 in ESI) which was sufficiently low for the detection of Zn^{2+} in many chemical and biological systems. The association constant (K_a) of

PDP-1 with Zn^{2+} were determined by using nonlinear least-squares fit analysis[29] of the ratio of the emission intensity at selected wavelengths in buffered solutions. It was found to be 4.4×10^{4} mol L^{-1}. The ^{1}H NMR spectrum Of PDP-1 in the presence of Zn(II) in DMSO-d$_6$ was measured(figure-S8 in ESI). After the addition of Zinc ions most of the protons of pyrene rings shifted to downfield and the methylene protons are split in four different signals. It clearly signifies that the smashing of the stacking-pyrene rings current and the change in the conformation after complexation of Zinc ions[30]. the influence of pH for the fluorescence of PDP-1was studied. At acidic pH (pH 1-5), the complete disappearance of the emission band at 515 nm with a sharp increase in emission at 435nm clearly indicates the disturbance of π- π stacking. Whereas in neutral and basic pH there is no appreciable change in fluorescence of PDP-1.

The PDP-1+Zn^{2+} complex showed the fluorescence band maxima at 412 and 470 nm. During the addition of pyrophosphate ions to the solution of PDP-1+ Zn^{2+} complex in HEPES buffer (10 mM HEPES, pH=7.4, H_2O: CH_3CN= 30:70). The emission maxima at 470 nm completely disappeared, and a new emission band at 515 nm emerged during addition of ppi ions

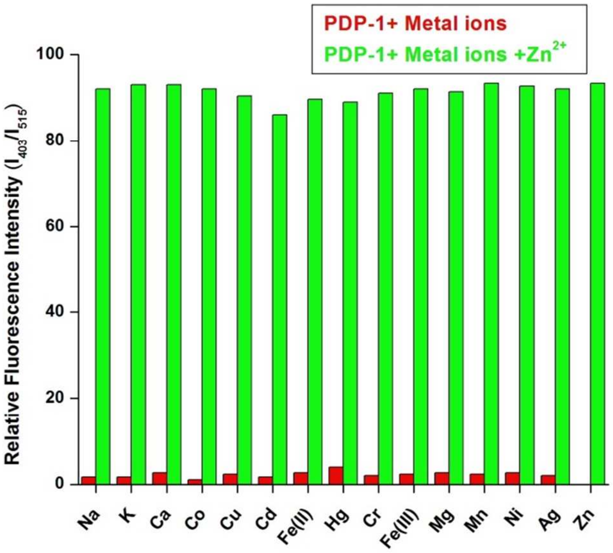


Figure-4: Fluorescence response (I/I0) of PDP-1 (10 µM) in CH_3CN/H_2O (30:70 v/v) to 10 µM of various tested metal ions (green bar) and to the mixture of 10 µM of tested metal ions with 10 µM Zn^{2+} ion (red bar).

Addition of pyrophosphate resulted in the change of fluorescence from blue to green. In order to know the selectivity of PDP-1+ Zn^{2+} complex among the anions such as PO$_4^{3-}$, F$^-$, Cl$^-$, Br$^-$, I$^-$, NO$_3^-$, NO$_2^-$, OAc$^-$, PF$_6^{-,}$ ClO$_4^-$, HPO$_4^{2-}$ and P$_2$O$_7^{4-}$, the pyrophosphate anion alone shows the fluorescence change.(figure-5,6,7). The fluorescence change after the addition of pyrophosphate ion with PDP-1+ Zn^{2+} complex can be accounted by the strong interaction of pyrophosphate ion with Zn^{2+}. The exchange of Zn(II) in PDP-1+ Zn^{2+} complex by the pyrophosphate ion leaving the probe PDP-1 in solution. This was further confirmed by ESI-MS analysis after addition of pyrophosphate ion with PDP-1+ Zn^{2+} complex showing the peak at m/z 577.43(PDP-1+H+) (Figure S5 in ESI).

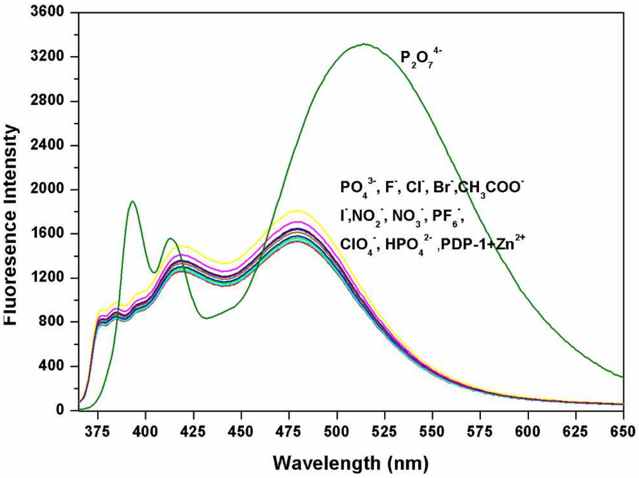


Figure-5: Fluorescence spectra of PDP-1+Zn^{2+}(1×10^{-5} molL-1) in the presence of PO4^{3-}, F$^-$, Cl$^-$, Br$^-$, I$^-$, NO$_3^-$, PF$_6^{-,}$ ClO$_4^-$, HPO$_4^{2-}$ and P$_2$O$_7^{4-}$,(10 µM) in aqueous HEPES buffer (10 mm HEPES pH 7.4) containing CH_3CN-H_2O (30:70). Excitation was performed at 350 nm

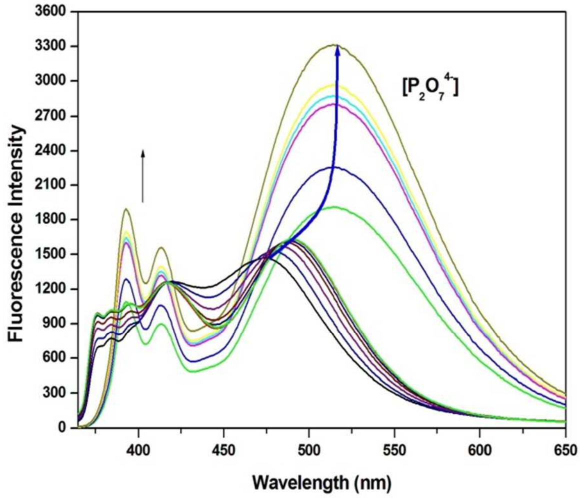


Figure-6: Fluorescence emission spectrum of PDP-1+Zn^{2+} (1×10^{-5} molL^{-1}) upon addition of pyrophosphate ions (0-100 equiv) in aqueous HEPES (10 mM HEPES, pH=7.4, H_2O: CH_3CN= 30:70).

DFT Calculations:

We also performed the DFT and time-dependent DFT by the Gaussian 03 program[31] to make clear the changes in the electronic properties. As we know, the highest occupied molecular orbital (HOMO) lowest unoccupied molecular orbital (LUMO) gap and the electronic distributions may be used to clarify the changes in the fluorescent properties with metal cations coordination and detect the ratiometric response to metal cations [32]. PDP-1 and PDP-1 + Zn^{2+} structures were optimised using B3LYP/6-31G, LANL2DZ methods respectively. The optimized geometries are shown in Figure 8. The optimised geometries show that the distance between the two pyrene units in PDP-1 is 4.537Å (C31-C12). It is clearly showing that the possibility of π-π stacking between the pyrene rings. Whereas in PDP-1+Zn^{2+} the distance is changed into 10.230Å (C31-C12), there is no possibility of effective π-π stacking in pyrene units.

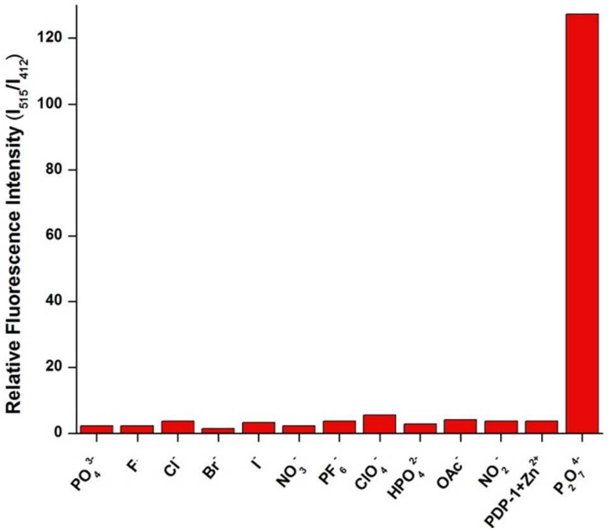


Figure-7: Bar chart representation of fluorescence response of PDP-1+Zn^{2+}(1×10^{-5} molL-1) in the presence of PO4^{3-}, F^-, Cl^-, Br^-, I^-, NO_3^-, PF_6^- , ClO_4^-, HPO_4^{2-} and $P_2O_7^{4-}$,(10 µM) in aqueous HEPES buffer (10 mm HEPES pH 7.4) containing CH_3CN-H_2O (30:70). Excitation was performed at 350 nm

In order to assess the practical utility of the probe PDP-1,PDP-1+Zn(II) ensemble for Zn(II) and pyrophosphate ions detection respectively in drinking water, tap water and river water samples was further carried out. In order to remove insoluble substances water samples were first filtered. All the samples with or without addition of zinc and pyrophosphate ions at different concentration levels of 0, 50 and 100 µg were analysed by probe PDP-1 and PDP-1+Zn(II) ensemble (Table S1 and S2). The experimental results show that PDP-1 and PDP-1+Zn(II) ensemble is able to measure the concentrations of Zinc and pyrophosphate ions respectively with virtuous recapture. These results indicate the suitability and applicability of the probe PDP-1 for the detection of Zn^{2+} and pyrophosphate ions from real samples.

To account on the variation of fluorescence intensity upon the addition of Zn^{2+} to PDP-1, we investigated the frontier molecular orbitals of PDP-1 and its Zn^{2+} complexes. In PDP-1 the HOMO shows that most electron density resides in one of the pyrene moiety and the LUMO on imine incorporating disulphide (spacer) unit whereas in PDP-1 + Zn^{2+} HOMO resided on sulfur unit, LUMO on both of the pyrene units (Figure-9).

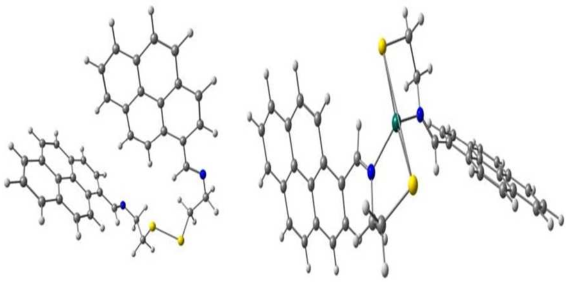


Figure-8: Energy Optimized geometry of PDP-1 and PDP-$Zn^{2+}$by B3LYP/6-31G and LANL2DZ method respectively.

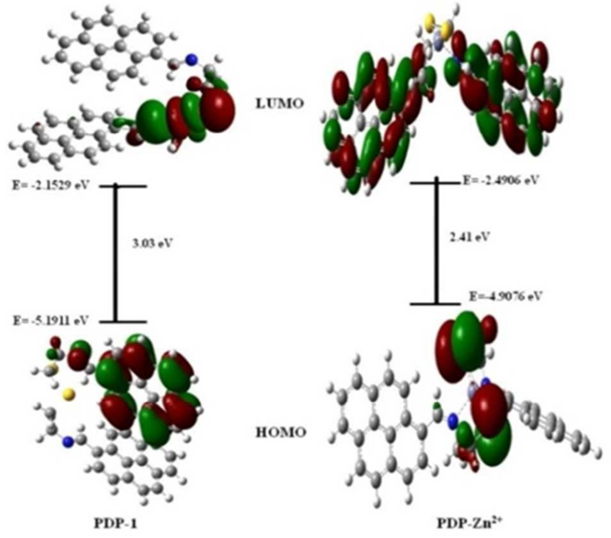


Figure-9: Frontier molecular orbital diagram of PDP-1 and PDP-1 + Zn^{2+}

HOMO and LUMO on PDP+Zn^{2+} shows a remarkable difference from the PDP-1. The excited states of PDP-1 can be stabilized by π-π interactions between the pyrene units whereas in PDP+Zn^{2+} which is not possible. It can be further evidenced by the HOMO-LUMO transition in PDP-1. In case of PDP +Zn^{2+} HOMO -1 - LUMO +1 transition shows that the presence of π-π interactions. It is clear that the excimer fluorescence from PDP-1 due to the existence of T-shaped stacked pyrene moieties. The emission band at 515 nm originates from the static excimer of pyrene units. These results clearly suggest that the ratiometric fluorescence change is due to conformational change from T-shaped stacked pyrene to non- stacked one after the appendage of zinc ions. In order to prove the conformational change of PDP-1 upon binding to Zn^{2+} but no other cations (figure - S11 in ESI) .The DFT calculations were carried out with PDP + Cu(II) ions. The optimized geometry of PDP + Cu(II) showed the distance between two pyrene rings is 5.85 Ǻ whereas in the case of PDP-Zn^{2+} the inter pyrene ring distance is 10.23 Ǻ . Hence PDP-1 showed the fluorescence change during the addition of zinc ions by change in distances between two pyrene rings.

Conclusions

In summary, we have designed a new pyrene derivative and synthesised by a single step convenient reaction. It shows a ratiometric fluorescent behaviour due to Excimer /monomer switching towards Zn^{2+} ions and pyrophosphate in aqueous acetonitrile solution. This system exhibits a novel detection of Zinc ions and pyrophosphate through interchanging of the excimer emission and monomer emission of pyrene.

Materials and Methods:

Synthesis of PDP-1:

0.25 g (1.085 mmol) Pyrene-1- carboxaldehyde is mixed with 0.120 g (0.5425 mmol) of Cystamine hydrochloride in ethanol. It was refluxed in the presence of Triethyl amine 0.35 g (1.5 mmol) for 8 hours under nitrogen atmosphere. On cooling to room temperature the desired compound was precipitated, after recrystallization with dichloromethane PDP-1 was obtained as pale yellow solid. Yield: 79 %, (Mp: 196 °C) 1H-NMR ($CDCl_3$, 300 MHz): 9.303 (s, 2H), 8.741-8.710 (d, 2H, J=9 Hz), 8.510-8.401 (dd, 2H, J=3.3 Hz) 8.308-8.287 (m, 6H), 8.119-7.902 (m, 8H), 4.269-4.225 (t, 4H, J= 6.6 Hz), 3.290-3.263 (t, 4H, J= 6.6 Hz). 13C-NMR ($CDCl_3$-75 MHz): 161.45, 132.80, 131.07, 130.40, 129.76, 128.57, 128.45, 128.19, 127.26, 126.20, 125.942, 125.74, 125.63, 124.78, 124.65, 124.43, 122.37, 61.07, 40.04. ESI-MS: m/z 577.42 [$(M+H)^+$] Calculated: (576.72). C38H28N2S2: Calculated C, 79.13; H, 4.89; N, 4.86. Found: C, 78.96; H, 4.49; N, 4.72.

Synthesis of PDP-1 + Zn(II) complex.

2 mmol of PDP-1 in Chloroform solution was added to the Ethanolic solution of $ZnCl_2$ (2.5mMol). During the addition of Zinc ions the colour turns to dark yellow, the stirring continued for 30 minutes. After evaporation of the solvent yielded dark yellow solid. ^{1}H-NMR (DMSO-d^6, 300 MHz): 9.427(s,2H),, 9.271-9.255(m,2H),,8.761-8.730,8.499 (m,16H),7.275-7.260 (t,3H), 4.183-4.144(t,2H), 3.070-3.051(t,2H), 3.306-3.284(t,2H),3.481-3.393(t,2H). Molecular formula: $C_{38}H_{28}Cl_2N_2S_2Zn$.

Acknowledgements:

GS thanks UGC for senior research fellowship. GS, TA and DC acknowledge DST– IRHPA, FIST, PURSE for instrumental facilities and funding. We thank Prof. S. Krishnaswamy, School of Biotechnology for providing the access to spectrofluorimeter facility.

Notes and references

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†Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/b000000x/

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