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A supramolecular strategy for ratiometric luminescence sensing of nitroaromatic explosives in water

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In the present work we have demonstrated a supramolecular approach for ratiometric luminescence sensing of nitroaromatic explosive compounds (NAEs) by employing a red luminescent Eu(III) complex and a green luminescent Pt(II) complex with in a Triton X-100 surfactant based micellar host in water. The Eu-complex gets entrapped inside micellar core whereas the Pt-C2 remains grafted on the surface of the spherical micelles due to their structural amendments and thus facilitates preferential interaction of Pt-C2 with NAE molecules mainly through π–π interaction. The presence of explosive traces quenches the green emission (508-545 nm) of Pt-complex whereas the red emission (614 nm) of Eu-complex remains unaffected. The strategy demonstrates first report of two independent luminophore based ratiometric sensing in water using a micellar host.

Keywords: Explosive, Ratiometric, Photoluminescence, Micelle, Supramolecular

The use of explosive materials by military personals, separatist organizations, and by respective agencies for constructing roads in hilly region or extracting valuable materials from mine have a grave consequence of ground water and soil contamination with explosive traces. The majority of these explosive traces are organic nitro compounds having adverse effect on marine life, soil microorganisms and human health.[1,2] Thus, trace level detection of these compounds, especially polynitro-aromatic molecules e.g., Dinitrotoluene (DNT), Trinitrotoluene (TNT) and Trinitrophenol (TNP) with high degree of precision is extremely important. Luminescence based technique is one of the most popular and advantageous method for detection of explosive molecules due to its extremely high sensitivity, easy portability, quick response and inexpensive processing cost.[3-9] Ratiometric luminescence-based technique is advantageous than a single luminophore based turn on/off strategies due to high sensitivity and inherent reliability, which reflect the self-calibration provided by monitoring two (or more) spectral signatures.[9-19] It is important to note that luminescence-based detection of explosives in organic solvent (lipophilic) has been explored extensively, as compared to those in aqueous medium due to ease of synthesizing lipophilic fluorophores (probes). The luminescence-based strategies to detect explosives in aqueous solutions include the use of hydrophilic fluorophores[11] confining lipophilic fluorophores in aqueous micellar media[20-23] and designing amphiphilic fluorophores based micellar structures.[24]

The use of micellar media for luminescence-based detection of analytes in aqueous media has been studied in recent years with major focus on metal ions detection[25-31] and lately the strategy has also been extended to detect explosive molecules.[20-24] For example, Zhao and coworkers reported using micelles to realize the dissolution of a nonpolar fluorescent sensor in aqueous solution for the detection of Hg^{2+} ions.[28], Bandyopadhyay et al. reported anionic micelle induced acridine orange based sensitive detection of Cu^{2+} in aqueous medium[25]. For the sensing of NAEs, pyrene derivatives have mainly been used in a micellar media where both pyrene and NAE molecules hydrophobically extracted to micellar core and results luminescence quenching through photo induced electron transfer (PET process).[20-22] A recent work by us has also demonstrated the use of a new pi electron rich Pt-complex appended on micellar surface for trace level detection of nitroaromatic explosives in aqueous media.[23] It is to be noted that the use of surfactant assemblies has brought many other advantages, including enhanced quantum yield and stability, less dependence on synthetic efforts, and increased sensitivity. All previous micelle-based

sensing reports have been performed using a single luminophore with monitoring of a single peak parameter. In the present work, we have demonstrated a new approach based on ratiometric emission intensities of two luminophores for explosive detection in aqueous media by employing a micellar host. We have used a Pt-CNN-$C_{12}H_{25}$ complex (Pt-C2) and a Eu-(Phen)$(DBM)_3$ complex (Eu-C1) as green and red luminophores, respectively and a neutral surfactant (Triton X-100) based micelle above its critical micellar concentration for successful demonstration of ratiometric luminescence sensing of NAEs in water.

Materials and Methods

Chemical reagents and instruments

All the starting materials and solvents were purchased from commercial sources (Sigma Aldrich Chemical Company, India and Finer Chemicals, India) and used as received. Milli-Q water and spectroscopic grade solvents were used for all measurements. Fourier-transform infrared (FTIR) spectra were measured on a JASCO FT-4600 spectrophotometer by using KBr pellet of samples. Solution phase optical absorption spectra were recorded on a JASCO V-670 spectrophotometer by using a quartz cell with optical path length of 1 cm. Steady state photoluminescence (PL) spectra were recorded using a JASCO FP-6500 fluorophotometer by using a quartz cell with optical path length of 1 cm. All solution phase PL measurements were carried out at RT (room temperature) and without removing dissolved O_2. The absolute photoluminescence quantum yield (PLQY) was determined by using a Hamamatsu Photonics C9920-02G instrument with Xe lamp as light source. Electrospray (ESI) mass spectra were recorded by using a micromass LCT instrument. Dynamic light scattering (DLS) based determination of hydrodynamic diameter (D_h) of micellar structures were performed using a Malvern DLS instrument (Zetasizer Nano ZSP model). Time resolved photoluminescence lifetime (decay profiles) measurements of the luminophores in various media were carried out by using a picosecond time-correlated single photon counting (TCSPC) instrument (Edinburgh Instruments Ltd, Lifespec II model). The IBH DAS 6.0 software was used to analyze the lifetime decays by the iterative reconvolution method.

Synthesis of the Eu-C1 and Pt-C2 complexes and micellar adducts

The Pt luminophore (Pt-C2) was synthesized by following a reported literature method[23] (Supplementary Data, Figs S1-S3). For the preparation of Eu-C1 complex, a previously reported method was followed with little modifications as briefed herewith.[32] Europium chloride ($EuCl_3 \cdot 6H_2O$, 366 mg, 1 mmol), 1,3-diphenyl-1,3-propanedione (673 mg, 3 mmol) and 1,10-Phenanthroline (180 mg, 1 mmol) were taken in a 50 mL round bottom flask and 50 mL of methanol was added to it. The pH of the solution was adjusted to 6.5 by adding few drops of aqueous KOH solution and then the solution was stirred at 65 °C for 24 h under a reflux condenser. Later the solution was cooled to RT, filtered and dried under vacuum using a Rota evaporator. The final product was purified by silica gel column chromatography using methanol-DCM (1:1) as eluent (Yield, 75 mg, 68 %, m/z 1002) (Supplementary Data, Figs S4-S7).

A 1 mM Triton X-100 solution was prepared by dissolving 647 mg of Triton X-100 (mol. wt. 647) in 100 mL water. The metal luminophore based three types of micellar adducts were prepared by dissolving (1×10^{-5}) mole of either Eu-C1 (10 mg) or Pt-C2 (6.2 mg) separately or together to a 1 L 1 mM Triton X-100 solution in water at RT and kept under stirring in the dark for 24 h.

Luminescence sensing studies

All solution phase steady state luminescence studies were performed by a JASCO FP-6500 fluorophotometer using a capped quartz cell with path length of 1 cm. The common excitation wavelength of 410 nm was fixed by considering the excitation spectra of Eu-C1 and Pt-C2 complexes and was used in all studies. The photoluminescence studies in organic solvent (DCM) was performed by taking a (1×10^{-5}) M concentrations of either of the complexes or together followed by incremental addition of TNP in the range of 0.01 to 0.06 µmol. Similarly, we took 2 mL aqueous micellar luminophore adducts in a cuvette and incremental addition of TNP/TNT/DNT (0.01 to 0.06 µmol) was carried out to record PL titration spectra. The Limit of detection (LOD) for different luminescence titrations were calculated by considering signal to noise ratio of 3.

Results and Discussion

Photo-physical properties of Eu-C1 and Pt-C2 complexes

The metal complex based luminophores selected for this study possesses distinct spectral signatures

and structural specificity. The Eu-Cl is a neutral lipophilic molecule soluble in common organic solvents with broad absorption band spanning entire UV range (300 to 450 nm), sharp excitation spectra with λ_{max} at 410 nm and characteristic sharp lanthanide emission spectra. The red luminescent Eu-C1 complex showed sharp laporte forbidden emission peaks at 614 nm ($^5D_0 - {}^7F_2$), 590 nm ($^5D_0 - {}^7F_1$), and 577 nm ($^5D_0 - {}^7F_0$) with long lifetime ($\tau \sim 36\mu s$) value indicating phosphorescent emission characteristics (Fig. 1b and Table 1). On the other hand, the absorption spectra of Pt-C2 complex in DCM exhibit intense absorption bands from 250 to 420 nm with high molar extinction coefficients (Fig. 1a and Table 1). The solution phase steady state excitation and emission spectra of this complex shows characteristic excitation peaks in the range of 340 to 400 nm and emission peaks at 514 and 546 nm along with a weak emission band at 580-600 nm in DCM at 298 K. The origin of the emission peak for Pt-C2 is attributed to ^{3}LMCT i.e., $\pi^*(L) \rightarrow d\pi(Pt)$ transition. The detail photophysical data for both the complexes are summarized in Table 1. Since we wanted to demonstrate the use of lipophilic luminophores in water using micellar host, we first scrutinized the solubility parameters and spectral behavior of these two complexes in water using Triton X-100 as surfactant. It was ascribed that, the lipophilic Eu-C1 complex will be hydrophobically extracted to the core of the micelle and long hydrophobic aliphatic chain-based Pt-luminophore will remain grafted on the surface of the micelle thus forming a unique luminophore–micelle adduct in water (cf. Scheme 1). The surface grafting of Pt-C2 complex may be ascribed due to the incorporation of $-C_{12}H_{25}$ aliphatic chain inside the hydrophobic micellar core and thus keeping the partial ionic Pt(II)C^N^N head groups exposed to the surface of the micelle. A 1 mM 1 L aqueous solution of triton X-100, i.e., 2 times its critical micelle concentration was taken and 10 mg of Eu-C1 (1×10^{-5} mole) and 6.2 mg of Pt-C2 (1×10^{-5} mole), respectively were added to it separately with constant stirring in dark for overnight at room temperature. In a similar way same quantity of both luminophores were added to the same amount of surfactant solution. The emission spectra of all these three solutions denoted as Micelle-Eu-C1, Micelle-Pt-C2, and Micelle-Eu-C1-Pt-C2 are shown in Fig. 1c. It is to be noted that the emission characteristics including peak width and peak positions of Eu-C1 remains identical in both DCM and aqueous micelle medium although a little enhancement (1.1 times) of emission intensity was observed in micelle than in DCM. This enhancement could be ascribed due to vibration rigidity that it gains inside micellar core as compared to in DCM bulk phase. The peak positions,

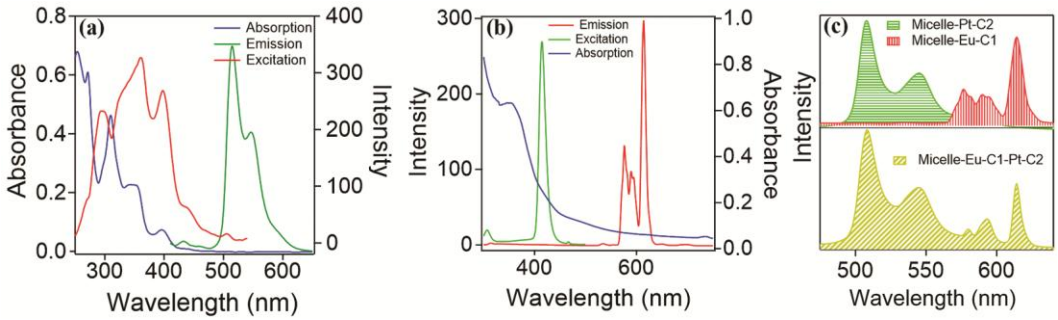
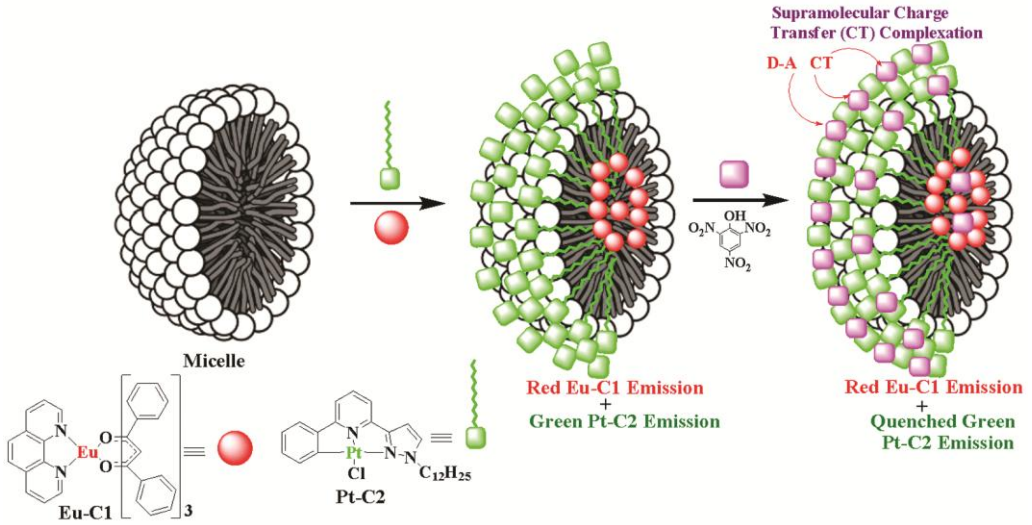


Fig. 1 — (a) Absorption, emission and excitation spectra of Pt-C2 complex in DCM at RT, (b) absorption, emission and excitation spectra of Eu-C1 complex in DCM at RT and (c) Luminescence spectra of aqueous Triton X-100-Eu-C1 adduct and Triton X-100-Pt-C2 adduct (above) and the luminescence spectrum of Triton X-100- Eu-C1-Pt-C2 adduct (below)

Table 1 — Photophysical data for Eu-C1 and Pt-C2 in DCM and in micellar media at room temperature

Compound	Medium	λ_{abs} (nm)	ε (dm^3 cm^{-1} L^{-1})	λ_{em} (nm)	FWHM (nm)	Φ (%)	τ_0
Eu-C1	DCM	250-400	2.1×10^3 (355 nm)	577, 590, 614	10	0.1	36 μs
Pt-C2	DCM	310, 355, 396	1.5×10^4, 7.2×10^3, 2.4×10^3	514, 546	54	9	45 ns
Triton X-100- Eu-C1	H_2O	–	–	577, 590, 614	10	0.1	28 μs
Triton X-100-Pt-C2	H_2O	–	–	508, 545, 580–585 (band)	45	17	50 ns



Scheme 1 — The chemical structures of luminophores Eu-C1 and Pt-C2 along with the schematic model showing two luminophores–micelle adduct with their preferential entrapment position responsible for ratiometric luminescence quenching upon interaction with TNP

peak width and emission intensities of Pt-C2 complex in DCM and in aqueous micelle media was thoroughly scrutinized and are shown in Figs 1a and 1c. As can be seen compared to DCM, the emission peaks of Pt-C2 showed ~ 9 nm blue shifted peaks with ~5 nm narrow FWHM value in micellar media (Table 1). The emission intensity was also found to be 1.3 times higher in micelle media as compared to DCM. We propose that the grafting of Pt-luminophores could substantially reduce vibrational relaxation and hence the vibrionic coupling-based broadening and non-radiative electronic transition is suppressed.

PL titration of TNP with Eu-C1 and Pt-C2 independently in DCM and water

The efficiency of both complexes for luminescence quenching based detection of TNP was first evaluated through steady state photoluminescence titration experiments in both DCM and in water using a micellar host. In actual experiment a 10^{-5} molar DCM solution of Eu-C1 or Pt-C2 was taken and its luminescence spectrum was recorded. Then TNP was incrementally added to the cuvette containing either Pt or Eu complexes and each spectrum was recorded. As shown in Fig. 2a we could see gradual decrease (quenching) of emission intensity with incremental addition of TNP to a DCM solution of Eu-C1. The Stern-Volmer constant (K_{sv}) calculated from the respective Stern-Volmer plot (Fig. 2c) is 2.4×10^3 M^{-1} which is a moderate value and the detection limit obtained is 0.1 μM. In the next experiment, the Eu-C1 was dissolved in water by using Triton X-100 based micellar host by maintaining the same concentration. As expected, the hydrophobic Eu-C1 gets solubilized in water through hydrophobic extraction by the micellar structures and remains entrapped in the core of the micelle. Luminescence titration of the obtained Eu-C1 entrapped Triton X-100 micelle solution with aqueous TNP solution was performed and the corresponding luminescence spectra are shown in Fig. 2b. Interestingly we observed very negligible quenching of emission intensity and the calculated Stern-Volmer constant (K_{SV}) is found to be 300 M^{-1} (Fig. 2c). Thus, the obtained K_{SV} value for Eu-C1 is 8 times lower than the corresponding value obtained in DCM as solvent for luminescence quenching based titration with TNP. The result could be attributed to the fact that in micellar environment, water soluble TNP molecules present in the bulk solvent could not interact with the Eu-C1 complex entrapped inside the core. On the other hand, the electron-rich Pt-C2 luminophore interacts very efficiently with a range of electron deficient nitroaromatic explosives in both organic solvent (DCM) and in water through solubilization by a micellar host. The room temperature luminescence titration spectra of a 10^{-5} M DCM solution of Pt-C2 with incremental addition of TNP was again performed and as seen in Fig. 3a, we could find gradual decrease of emission intensity. The corresponding Stern-Volmer graph was plotted (Fig. 3c) and K_{SV} value obtained from the graph calculated is 7.5×10^3 M^{-1}. The sensing mechanism involves the quenching of Pt(II)-based luminescence

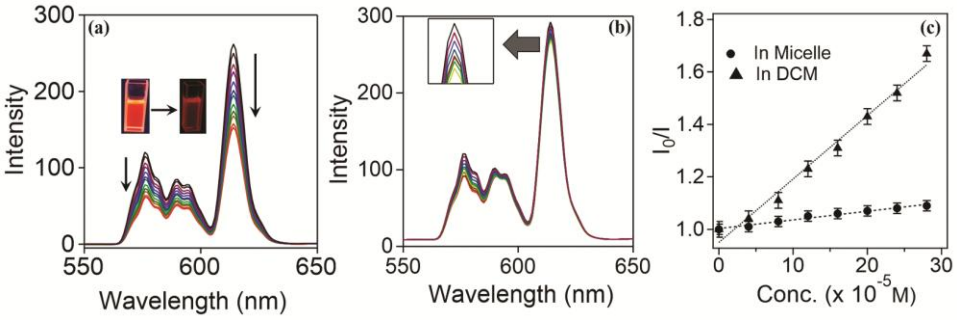


Fig. 2 — (a) PL titration spectra of Eu-C1 in DCM at 298 K with various amount of TNP, (b) PL titration spectra of Triton X-100-Eu-C1 in water at 298 K with various amount of TNP and (c) Stern-Volmer plots obtained from the PL titration spectra of Figures (a) and (b)

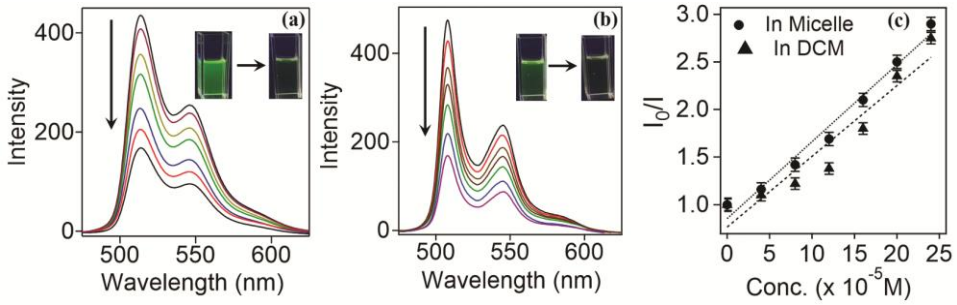


Fig. 3 — (a) PL titration spectra of Pt-C2 in DCM at 298 K with various amount of TNP, (b) PL titration spectra of Triton X-100-Pt-C2 in water at 298 K with various amount of TNP and (c) Stern-Volmer plots obtained from the PL titration spectra of Figs (a) and (b)

due to supramolecular charge transfer (CT) process originated from Pt(II)C^N^N-antenna to the electron deficient nitroaromatic explosives. In next experiment the Pt-C2 luminophore was solubilized in Triton X-100 based aqueous micellar medium and then an aqueous TNP solution was added to it gradually to see the extent of luminescence quenching (Fig. 3b). The K_{SV} value obtained from the corresponding Stern-Volmer plot (Fig. 3c) is 1.1×10^4 (for Triton X-100), which is 1.5 times higher than KSV value obtained in DCM. We choose to use a neutral surfactant, Triton X-100 due to its superiority over cationic or anionic surfactants (CTAB or SDS) due to interference of charged surfactant head groups with neutral TNP molecules.[23] The above results clearly demonstrate that structural amendments of two luminophores is responsible for their differential entrapped positions in micellar structure. The long hydrophobic chain-based Pt-C2 gets entrapped on surface of the micelle, whereas the near symmetrical hydrophobic Eu-C1 gets entrapped in the core of micelle.

Explosive detection through ratiometric fluorescence in water using micellarhost

Ratiometric luminescence-based technique is advantageous than a single luminophore based turn on/off strategies due to high sensitivity and inherent reliability, which reflect the self-calibration provided by monitoring two (or more) spectral signatures.[9-19] We were particularly interested to realize a ratiometric luminescence sensing strategy using these two luminophore together in a particular solvent media. However, using a DCM solvent for two luminophores together, we tried to do luminescence titration by adding increasing concentrations of TNP in the range of 0.01 to 0.06 µmol (Fig. 4a). But as observed in previous experiments we could see quenching of emission peaks for both Eu-C1 (at 614 nm) and Pt-C2 (at 514-550 nm), thus excludes any selectivity of either of these two complexes when they present together. In next set of experiments, we decided to solubilize two luminophores together in the Triton X-100 based aqueous micellar media. We envisioned that due to their structural difference, Eu-C1 will be entrapped inside micellar core whereas the Pt-C2 luminophore will remain grafted on micellar surface. The resulting dual luminophore micellar adduct could be used for ratiometric luminescence sensing based detection of TNP where the surface exposed Pt-C2 will interact with TNP but Eu-C1 present in the core cannot interact with TNP (Scheme 1). The luminescence titration experiment was performed where an incremental amount of aqueous TNP solution was added to the Aqueous Micellar-Eu-C1-Pt-C2 adduct solution in a cuvette.

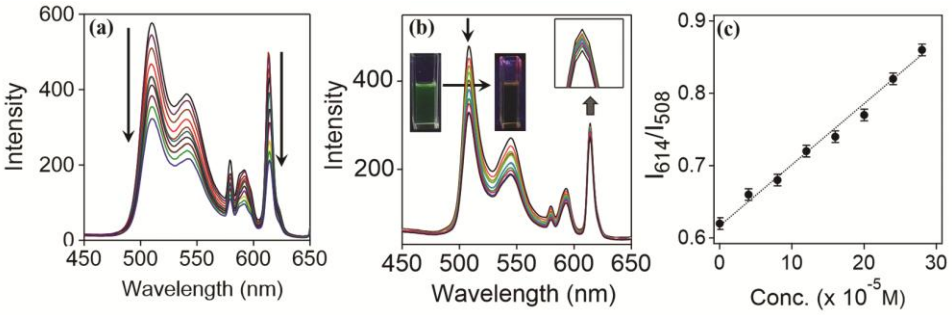


Fig. 4 — (a) PL titration spectra of Eu-C1+Pt-C2 in DCM at 298 K with various amount of TNP, (b) PL titration spectra of Triton X-100-Eu-C1-Pt-C2 in water at 298 K with various amount of TNP and (c) The ratiometric emission intensity plot of I_{614}/I_{508} vs. concentrations of TNP obtained from the luminescence titration plot, i.e. Fig. 4b

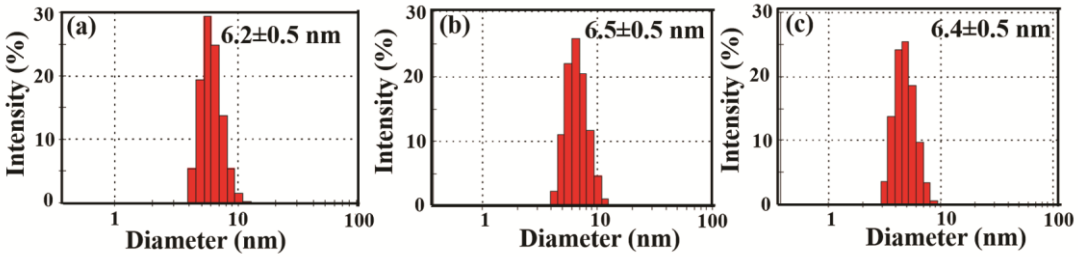


Fig. 5 — Hydrodynamic diameter (Size distribution histograms) of Triton X-100 based micellar structures (a) 1 mM Triton X-100 solution in water, (b) Triton X-100-Eu-C1-Pt-C2 adduct and (c) Triton X-100-Eu-C1-Pt-C2+TNP

The titration experiment result in gradual diminishing of luminescent intensity at 508 and 540 nm peaks originated from Pt-C2 but, the sharp peak at 614 nm originated from Eu-C1 remains almost constant (Fig. 4b). This result unequivocally supports the general hypothesis depicted in Scheme 1, a longer aliphatic chain based luminophore and another small hydrophobic luminophore could be used for ratiometric luminescence sensing for water soluble analytes in a micellar host support. We believe in molecular level the electron poor planar TNP molecules get sandwiched between two planar Pt(II)C^N^N head groups situated on the micellar surface, which in turn promotes photo induced electron transfer from Pt-C1 to TNP and results luminescence quenching. The movement of TNP molecules gets restricted on micellar surface through the formation of above supramolecular donor-acceptor adduct, thus few rare molecules are able to penetrate to the core of micelle to interact with Eu-C luminophore. Also, hydrophobic micellar core repeals the hydrophilic TNP analytes and further protects the luminescence of Eu-C1 to get diminished through interaction with it.The plot of comparative (ratiometric) emission intensity at two different wavelengths (I_{614}/I_{508}) against concentration of TNP is shown in Fig. 4c.

To understand the stability of micellar structure, we have also measured the hydrodynamic diameters (D_h) of 1 mM Triton X-100 based micelle, its corresponding dual luminophore adduct (Micelle-Eu-C1-Pt-C2) and the same adduct after addition of TNP (Fig. 5). We could find that the measured hydrodynamic diameters remain almost similar for all three samples. More importantly we did not find any small fraction of higher D_h values in any of the micellar structures, especially in micelle-luminophore adducts implying the fact that the spherical micellar structures remain intact in all three samples measured here. The excited state lifetime of Eu-C1 and Pt-C2 luminophores in the micelle environment were also measured before and after the addition of TNP and the results are shown in Fig. 6. We could observe a substantial decrease of excited lifetime for Pt-C2 complex, as its average τ value decreases from 50 ns to 30 ns which implies its efficient interaction of TNP and photoinduced electron transfer event. On the other hand, the long-excited state lifetime of Eu-C1 decreases slightly as evident from reduction of its τ values from 36 μs to 28 μs after addition of TNP.

The PL titration experiments were also carried out for other two nitroaromatic explosives (DNT and TNT) with aqueous micellar-Eu-C1-Pt-C2 adduct

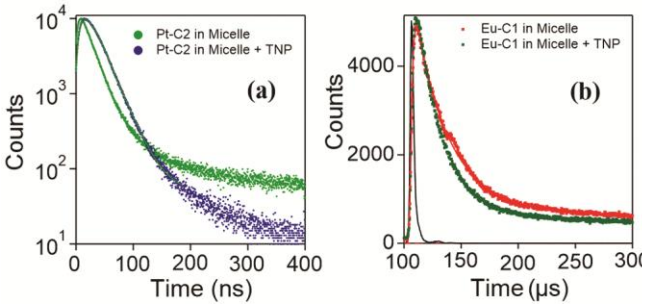


Fig. 6 — Excited state Lifetime decay traces for (a) Pt-C2 and (b) Eu-C1 luminophore in their micelle adduct form in water at RT (i.e., Triton X-100-Eu-C1-Pt-C2) with and without addition of TNP

solution under identical conditions (Supplementary Data, Fig. S8) and we could observe similar ratiometric luminescence quenching result. This is to be mentioned that, DNT and TNT are not directly soluble in water and hence a methanolic solution of these two compounds were diluted with water to make up the desired concentration in aqueous medium. The above two PL experiments univocally proves that our strategy of luminescent supramolecular adduct based ratiometric luminescence sensing of NAEs is very efficient. The limit of detection for TNP, TNT and DNT were calculated to be 50, 55 and 80 nM, respectively.

The mechanism of this sensing is π–π adduct formation between electron deficient nitroaromatic explosives and electron rich luminophores followed by photoinduced electron transfer from luminophore to NEA.[7] The hydrophobic extraction of NAEs to micellar core is not operative in our system as reported by other groups[20] due to efficient adduct formation between Pt-C2 and NAE on surface of the micelle. The possibility of Forster Resonance Energy Transfer as an operative mechanism[8] is also ruled out as we see that absorption profiles of NAEs and luminescence spectra of either Pt-C2 or Eu-C1 has no overlapping region (Supplementary Data, Fig. S9).

Conclusions

In summary, we have demonstrated a new strategy for ratiometric luminescence quenching based detection of aqueous nitroaromatic molecules by employing two separate luminophoresent trapped in a micellar host. Two metal complexes were used for this purpose with special spectral and structural amendments, i.e., (i) A green luminescent long alkyl chain based Pt(II)-CNN-$C_{12}H_{25}$ complex (Pt-C2) and a (ii)a red luminescent Eu(III)-(Phen)-$(TBA)_3$ complex (Eu-C1). In organic solvent, e.g., dichloromethane both the complexes showed luminescence quenching in presence of electron deficient nitroaromatic molecule (TNP) through supramolecular complex formation followed by photoinduced electron transfer (For Pt-C2, K_{SV} of 7.5×10^3 M^{-1}, for Eu-C1, KSV of 2.2×10^3 M^{-1}). However, in presence of neutral micelle (Triton X100) The Eu-C1 gets entrapped inside micellar core whereas the Pt-C2 remains grafted on the surface of the spherical micelles due to their structural amendments and thus facilitates preferential interaction of Pt-C2 with nitro aromatic explosive molecules through π–π interaction. The presence of explosive traces quenches the green emission (539 nm) of Pt-C2 (K_{SV} ~1.1×10^4 M^{-1}) whereas and the red emission (614 nm) of Eu-C1 remains unaffected (K_{SV} ~300 M^{-1}). The hydrophobic Eu-C1 gets entrapped inside micellar core whereas the presence of long hydrophobic chain of Pt-C2 ensures its insertion to the micellar core leaving Pt(II)C^N^N head group exposed towards the surface of the micelle. The sensing mechanism involves the quenching of Pt(II)-based luminescence due to supramolecular charge transfer process originated from Pt(II)C^N^N-antenna to the electron deficient nitroaromatic explosives. We believe that our proposed methodology could be generalized for ratiometric luminescence quenching based detection of analytes in aqueous medium.

Supplementary Data

Supplementary data associated with this article are available in the electronic form at http://nopr.niscair.res.in/jinfo/ijca/IJCA_59A(12)1814-1821_SupplData.pdf.

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