CHAPTER 3
Ultra-trace Detection of Nitroaromatics: Picric acid Responsive Aggregation - Disaggregation of Self-assembled p-Terphenyl-benzimidazolium based Molecular Baskets

Abstract

TRIPOD-TP molecules undergo self-assembly to form rod like structures in aqueous medium, as shown by field-emission scanning electron microscope, transmission electron microscope and dynamic light scattering studies. On gradual addition of picric acid (PA), these aggregates undergo aggregation - disaggregation process to complex morphological structures ($10^{-12} - 10^{-10}$ M PA) and spherical aggregates ($10^{-9} - 10^{-8}$ M PA). These spherical aggregates undergo further dissolution to well dispersed spheres between $10^{-7} - 10^{-6}$ M PA. During fluorescence studies, these aggregates demonstrate super-amplified fluorescence quenching (> 97%) in the presence of $10^{-5}$ to 0.2 equiv of the probe concentration, an unprecedented process with PA. The lowest detection limits by solution of TRIPOD-TP are $5 \times 10^{-13}$ for PA, $50 \times 10^{-12}$ M for 2,4-DNP, $200 \times 10^{-12}$ M for TNT and 1 nM for Cl-DNB. The paper strips dipped in the solution of TRIPOD-TP demonstrate quantitative fluorescence quenching between $10^{-17} - 10^{-6}$ M PA using front surface steady state studies and can measure as low as $2.29 \times 10^{-20}$ g/cm$^2$ PA.

Morphological changes in TRIPOD-TP on gradual additions of picric acid. Upper line = FESEM images; Lower line = TEM images (a, e) [PA] = 0 M; (b, f) [PA] = $10^{-12}$ M; (c, g) [PA] = $5 \times 10^{-8}$ M; (d, h) [PA] = $2 \times 10^{-7}$ M

3.1 Introduction

Nitro aromatic compounds (NACs) such as trinitrotoluene (TNT), dinitrotoluene (DNT), 1,3,5-trinitro-perhydro-1,3,5-triazine (RDX) and picric acid (PA), the main ingredients in the explosives, have attracted increasing attention for protection against threat of terrorism at entrance portals and other domestic situations such as in buses, trains, buildings etc. These NACs are also continuously being released into the environment during their commercial production or leaching from military waste sites, mining activity and space experiments. The degradation of these NACs leads to generation of reactive nitrogen oxide species, which readily react with biological macromolecules and cause formation of potent genotoxic and mutagenic metabolites. So, the detection of NACs at ultra low level has become an urgent issue in the interest of both national security and environmental protection.

The analysis of fluorescence signal for the detection of NACs has emerged as a prominent technique by virtue of its high detection sensitivity, selectivity and applicability in both solution and solid phase. The fluorescent conjugated polymers due to their excellent molar absorptivity, high quantum yields and amplified sensory responses have been found to be more advantageous as compared to small fluorescent molecules. Swager et al. have made number of reports on the trace detection of TNT and its commercialized product FidoXT has been applied in war zones.

In another approach, several fluorescent nanofibers obtained by self-assembly of simple molecular systems have been reported as sensing materials for the detection of nitro aromatic explosives. The effective $\pi-\pi$ stacking between these molecules provides ordered molecular organization and enables long-range exciton migration and their quick annihilation by the explosive quenchers. However, research work on NACs is mainly directed towards the detection of TNT and less attention has been paid to picric acid (PA) although its explosive power is superior to that of TNT.

So, it is of extreme importance to develop self-assembled molecular probes which can undergo aggregation or disaggregation process in the presence of picric acid to single molecular stage and thus can provide level for ultra-trace detections for picric acid. Further, the probes which can detect PA at less than nano or even pico molar concentrations from solutions or vapors are essential for practical applications for
detection of traces of PA. In the previous chapter, we have shown that tripodal system can encapsulate PA molecule selectively over other NACs. We envisaged that the replacement of biphenyl moiety with p-terphenyl moiety in the tripodal system will increase the depth of the cavity for encapsulation of NACs. If such tripodal systems can undergo aggregation, it may undergo more efficient exciton transfer from donor to acceptor molecule due to increased proximity of the fluorophores. This may result in increased sensitivity towards NACs.

So, we have designed and synthesized a 1-(p-terphenyl)-benzimidazolium based probe TRIPOD-TP which in water - DMSO (2%) undergoes aggregation - disaggregation modulated detection of picric acid (PA). Field-emission SEM, TEM and DLS studies demonstrate the formation of self-assembled rod like structures. In the presence of 10^{-12} to 10^{-6} M PA, these aggregates undergo complex aggregation - disaggregation process before their dissolution to well dispersed spheres. The blue emission of these aggregates undergoes super-amplified fluorescence quenching (> 97%) in the presence of 0.2 equiv of PA of the TRIPOD-TP concentration, both in the solution and solid state. TRIPOD-TP can detect 5 \times 10^{-13} M (500 fM) PA in solution phase and as low as 10 μl of 10^{-17} M (i.e. 2.29 \times 10^{-20} g/cm^{2}) PA using TRIPOD-TP coated paper strips. 2,4-Dinitrophenol, trinitrotoluene and 2,4-dinitrochlorobenzene can be detected but at much higher concentration. Other NACs do not show any response even at equivalent amounts.

3.2 Results and Discussion
3.2.1 Synthesis of TRIPOD-TP

Scheme 1: Synthesis of chemosensor TRIPOD-TP
1-(p-Terphenyl)benzimidazole (1) was synthesized by cuprous iodide and benzotriazole catalysed N-arylation of benzimidazole with 1-bromo-p-terphenyl (Scheme 1). The reaction mixture on usual work-up and chromatography gave compound 1 in Yold 60 %; m. p. 192 °C. The refluxing of solution of compound 1 and tribromide 2 in 3:1 ratio in acetonitrile at 90°C under N₂ gave TRIPOD-TP in 90% yield, m. p. 245 °C (Scheme 1). The structure of TRIPOD-TP was confirmed by spectroscopic techniques viz. ¹H NMR, ¹³C NMR and HRMS.

3.2.2 Selection of solvent for photophysical studies

In order to find the appropriate solvent medium for studying interactions of TRIPOD-TP with PA, the UV-Vis absorption and fluorescence spectra of TRIPOD-TP in DMSO and DMSO-water mixtures containing varied amounts of water were recorded. The absorption spectrum of TRIPOD-TP in DMSO-water (10%) gives broad maxima at 300 nm with a shoulder at 280 nm. On increasing the amount of water ratio upto 70 %, absorption spectrum does not undergo any significant change, but on further increasing the volume of water to 80 % or more, the absorption at 310 nm decreases sharply leaving maxima at 280 nm. The plot of I₀/I shows a regular but small increase in value with increase in ratio of water up to 80% and then I₀/I increases sharply and points to the aggregation of TRIPOD-TP in > 90% aqueous medium (Figure 1, 2a). The fluorescence spectrum of TRIPOD-TP in DMSO and aqueous media upto 90% water ratio gave emission maxima between 450 – 465 nm, but on increasing the amount of water ≥ 95%, the emission maxima was blue shifted between 402-410 nm. This further confirms the aggregation of TRIPOD-TP molecules in water ratio ≥ 95%.

![Figure 1](image1.png)  
Figure 1: The effect of increasing ratio of water on (a) absorbance and (b) emission spectra of TRIPOD-TP (5 µM)
In order to further ascertain the formation of aggregates, the dynamic light scattering (DLS) data (Figure 2b) of these solutions was recorded. In solutions containing less than 90% water, scattering of light was not observed. On increasing the amount of water to 95% or 98%, poly-dispersity of particles between 50-900 nm with Z-average 785 nm was observed. So, all the studies were performed in water – DMSO (98:2) mixture as solvent. The DLS experiment of solution of TRIPOD-TP showed the formation of aggregates between sizes of 100-200 nm and

![Figure 2: (a) The effect of ratio of water on I,I of the solutions of TRIPOD-TP (5 μM); (b) Distribution of different sized particles in poly-dispersed solution of TRIPOD-TP (10 μM, water – DMSO (98:2) on gradual addition of picric acid aliquots. P 40-100 nm; Q 100-200 nm; R 220-396 nm; S 531-712 nm; T > 800 nm. A = TRIPOD-TP (10 μM); B (A+10-10 M PA); C (A+10-9 M PA); D (A+10-8 M PA); E (A+10-7 M PA)](image)

530-712 nm, only. On addition of picric acid (10-10 M), the intensity of aggregates between 100-200 nm size was drastically decreased and aggregates with size > 800 nm
made significant (14.3%) contribution. On further increasing the concentration of picric acid to $10^{-9}$ and $10^{-8}$ M, the contribution of aggregates with > 800 nm size was further increased to 20 and 37%, respectively. However, on increasing the concentration of PA to $10^{-7}$ M, all these aggregates dissolved to the size less than 200 nm (Figure 2b). Therefore, self-assembled aggregates of TRIPOD-TP in water-DMSO (98:2) undergo aggregation-dissaggregation process on gradual addition of PA. The formation of these aggregates and their aggregation - disaggregation process in the presence of varied amounts of PA has been substantiated by field-emission scanning (FE SEM) and transmission electron (TEM) microscopic techniques.

3.2.3 Field-emission scanning electron and transmission electron microscopic studies

![Morphological changes in TRIPOD-TP on addition of various amounts of picric acid.](image)

**Figure 3**: Morphological changes in TRIPOD-TP on addition of various amounts of picric acid. (a) \([PA] = 0\) M; (b) magnified view of selected area in fig1(a); (c) \([PA] = 10^{-12}\) M; (d) magnified view of selected area in fig1(c); (e) \([PA] = 5 \times 10^{-11}\) M; (f) magnified view of selected area in fig1(e); (g) \([PA] = 10^{-9}\) M; (h) magnified view of selected area in fig1(g); (i) \([PA] = 5 \times 10^{-8}\) M; (j) magnified view of selected area in fig1(i); (k) \([PA] = 2 \times 10^{-7}\) M; (l) magnified view of selected area in fig1(k).
The Field-emission SEM image of drop-casted thin film of solution of TRIPOD-TP demonstrated self-assembly of TRIPOD-TP molecules into rod like structures (Figure 3a, b). Figure 3b shows the high porosity in these rod like structures which can capture small NAC molecules through electrostatic interactions and or hydrophobic π-π interactions with respective benzimidazolium and p-terphenyl units. TEM images of TRIPOD-TP (Figure 4a, b) also showed the formation of similar rod like morphology. In order to substantiate the amorphous or crystalline phases of these rods, the selected area electron diffraction (SAED) of these rods was performed inside the transmission electron microscope. The absence of any diffraction spots in SAED of these rods (Figure 5) clearly demonstrated their amorphous nature. The X-ray diffraction analysis of the solid TRIPOD-TP precipitated from DMSO-water (1:1) mixture showed a broad peak (Figure 6) and further supported the amorphous nature of the material. The increased resolution of these rods showed their formation by self-assembly of threads with ~ 1.5 nm diameter and 800-900 nm length. These threads could be further resolved into small dots in HRTEM. The energy minimized structure (Figure 7) of TRIPOD-TP was calculated using density functional theory at B3LYP / 6-31G basis set. The length and breadth of the TRIPOD-TP was found to be ~ 2.0 nm and 1.5 nm, respectively. By considering the length of the TRIPOD-TP molecule, it seems that each thread is formed by linear aggregation of TRIPOD-TP molecules where each dot represents one phenyl ring. These threads are separated by ~ 2.5 nm representing the width (1.5 nm) of TRIPOD-TP molecule. Significantly, earlier reported 1-(4-biphenyl)benzimidazolium based tripodal system discussed in previous chapter formed well organized basket like structure but did not undergo aggregation under similar conditions. As the hydrophobic p-terphenyl units are well known to cause the aggregation, in the present studies, the aggregation of the TRIPOD-TP molecules could be attributed to the presence of p-terphenyl moieties.
Figure 4: TEM images of (a,b) TRIPOD-TP (5 μM); (c) TRIPOD-TP (5 μM) + PA (10^{-12} M), (d) TRIPOD-TP (5 μM) + PA (10^{-9} M); (e,f) TRIPOD-TP (5 μM) + PA (2 \times 10^{-7} M) in H_2O-DMSO (98:2)

Figure 5: SAED pattern of TRIPOD-TP rods section

Figure 6: X-ray diffraction analysis of TRIPOD-TP powder
Figure 7: Energy minimized structure of TRIPOD-TP. Carbon (black spheres); nitrogen (blue spheres); hydrogen (white spheres). The structure has been optimized by DFT/B3LYP calculations and 6-31G basis set on TmoleX platform.

The drop-casted thin film of TRIPOD-TP solution possessing $10^{-12}$ M PA showed the beginning of the morphological change in rod like structures (Figure 3c, 3d) where both aggregation and disaggregation of these rods was apparent. This beginning of morphological changes in TRIPOD-TP is also evident in TEM image (Figure 4c). FESEM images of thin film containing $5 \times 10^{-11}$ M PA in TRIPOD-TP (Figure 3e, 3f) demonstrated the initial formation of spherical aggregates along with rod like structures. The film obtained from TRIPOD-TP containing $10^{-9}$ M PA (Figure 3g, 3h) showed almost complete conversion to spherical aggregates and this process was completed when concentration of PA was increased to $5 \times 10^{-8}$ M (Figure 3i, 3j) and spherical aggregates with diameter between 200-600 nm were formed. The formation of these spherical aggregates was also confirmed by TEM image (Figure 4d).

On further increasing the concentration of PA to $2 \times 10^{-7}$ M in TRIPOD-TP (5 μM) solution, FESEM image of the thin-films showed the drastic decrease in the concentration of spherical particles (Figure 3k and 3l). TEM image of similar film obtained on copper grid (Figure 3e, 3f) also confirmed the formation of spherical aggregates with 50-60 nm diameter which could undergo further aggregation to form larger size aggregates. The recording of SAED and XRD powder data on these spherical aggregates pointed to their amorphous nature (Figure 8, 9). On further increasing the concentration PA to $5 \times 10^{-6}$ M
Figure 8: SAED pattern of spherical aggregates of TRIPOD-TP + PA

Figure 9: X-ray diffraction analysis of TRIPOD-TP + PA

Figure 10: (a) The gradual decrease in FI on addition of aliquots of PA to the solution of TRIPOD-TP (5 μM, H₂O-DMSO; 98:2), λₐₓ = 290 nm; (b) plot of FI vs [PA]. Inset: Plot of FI vs [PA] showing sensitivity of the probe TRIPOD-TP towards PA in TRIPOD-TP (5 μM) solution, the formation of aggregates was not observed which is in agreement with no-scattering of light in DLS studies. This clearly shows that at 1 equiv. of PA, the interaction of PA with TRIPOD-TP is purely at molecular level and is in consonance with almost complete quenching (> 97%) of the fluorescence intensity of TRIPOD-TP in solution phase (Figure 10). Therefore, the overall mechanism of aggregation – disaggregation of TRIPOD-TP could be understood in the following manner. PA enters in to the rod like morphology of TRIPOD-TP and leads to breaking of the aggregate at that point. This results in further reorganisation of the newly formed aggregates leading to complex morphological structures. After addition of 10⁻⁷ to 10⁻⁶ M
PA, the dissolution of these aggregates to well dispersed spheres occurs which are finally dissolved to make homogeneous solution.

Moreover, the FESEM images of thin films of TRIPOD-TP obtained after addition of 1 nM concentration of TNT and Cl-DNB apparently retain the rod like morphology of the thin film of pure TRIPOD-TP. Therefore, rod like morphological structures of TRIPOD-TP is quite stable to TNT and Cl-DNB at concentrations < 1 nM. These results are in consonance with the poor fluorescence response of TRIPOD-TP solutions towards TNT and Cl-DNB, discussed in next section. On increasing the concentration of TNT to $10^{-6}$ M, the corresponding thin film shows the formation of spherical particles of 140-170 nm (Figure 11a). In case of $10^{-6}$ M Cl-DNB, the formation of mixture of 20-40 nm particles and platelet like structures is observed (Figure 11b). However, thin film of TRIPOD-TP obtained after addition of $10^{-10}$ M concentration of 2,4-DNP shows rods as well as spherical structures which changes to densely populated smaller particles at $10^{-8}$ M concentration (Figure 11c and 11d). Therefore, TRIPOD-TP undergoes morphological changes with respect to amount of various NACs which is in order PA > 2,4-DNP > TNT ~ Cl-DNB.

![Figure 11](image_url)

**Figure 11:** Morphological changes in TRIPOD-TP on addition of various amounts of (a) [TNT] = $10^{-6}$ M; (b) [Cl-DNB] = $10^{-6}$ M; (c) [2,4-DNP] = $10^{-10}$ M; (d) [2,4-DNP] = $10^{-8}$ M
3.2.4 UV-Vis and Fluorescence Studies

We hypothesized that TRIPOD-TP, due to its rod-like porous structure, could facilitate the binding process with PA and would also result in change of its optical properties. The UV-Vis absorption spectrum of TRIPOD-TP (5 µM, water-2% DMSO) exhibits absorption band at 270 nm ($\varepsilon = 65000$). On excitation at 290 nm, the solution of TRIPOD-TP (5 µM) gives $\lambda_{em}$ centered at 402 nm ($\Phi = 0.05$). The UV-Vis absorption spectrum of TRIPOD-TP does not show any significant change in its spectrum on addition of $10^{-10}$ M concentration of various (nitro)aromatic compounds, viz. phenol, 2-nitrophenol (2-NP), 4-nitrophenol (4-NP), 2,4-dinitrophenol (2,4-DNP), picric acid (PA), 4-hydroxybiphenyl (4-OHBP); 2-nitrotoluene (2-NT), 2,4-dinitrotoluene (2,4-DNT), dinitrobenzene (DNB), 2-chloronitrobenzene (2-CINB), trinitrotoluene (TNT); 3-chloronitrobenzene (3-CINB), 4-chloro-nitrobenzene (4-CINB), chlorodinitrobenzene (Cl-DNB); 2-nitroaniline (2-NA) and 2,4-dinitroaniline (2,4-DNA) (Figure 12a). Therefore,

![Figure 12](image)

**Figure 12:** (a) The UV-Vis spectrum of TRIPOD-TP (5 µM, H$_2$O-DMSO: 98:2), on addition of various NACs; (b) Effect of various NACs ($10^{-10}$ M) on the emission intensity of the TRIPOD-TP at 402 nm in water-DMSO (98:2). TRIPOD-TP (5µM); PA+ = TRIPOD-TP + picric acid; 2,4-DNP+ = TRIPOD-TP + 2,4-dinitrophenol; TNT+ = TRIPOD-TP + trinitrotoluene; Cl-DNB+ = TRIPOD-TP + chloro-2,4-dinitrobenzene

the formation of charge-transfer complex between TRIPOD-TP and nitro-aromatic compounds especially picric acid is ruled out. However, the emission spectrum of TRIPOD-TP, on addition of $10^{-10}$ M PA, results in 40% quenching in the fluorescence intensity whereas the addition of other closely related NACs such as 2,4-DNP, TNT and Cl-DNB ($10^{-10}$ M each) cause <1% change in the fluorescence intensity of TRIPOD-TP (Figure 12b). Even on addition of 0.1 equiv. (0.5 µM) of NACs viz., PA, 2,4-DNP, TNT
and Cl-DNB the respective percentage quenching of 88, 70, 39, 20 was observed. The other NACs had minimal effect on the fluorescence intensity of TRIPOD-TP solution. Therefore, at sub nano molar concentrations of NACs, TRIPOD-TP is highly selective and sensitive towards PA.

On gradual addition of aliquots of PA to the solution of TRIPOD-TP (5 μM, water - 2% DMSO), the fluorescence intensity at 402 nm was gradually quenched up to the addition of 0.5 μM of PA and on further addition of PA, no significant change in fluorescence intensity was observed (Figure 10). The plot of fluorescence intensity vs [PA] is non-linear and fluorescence intensity decreases exponentially with increase in concentration of PA. The present system is unique which shows the amplified quenching at < 0.1 equiv. of PA and its sensitivity outperforms previously reported PA chemosensors. In literature46-50, super-amplified quenching has been observed only at very high concentrations of NACs. The plot of I/I₀ vs. log[PA] demonstrates three steps of linear change between $5 \times 10^{-13} - 5 \times 10^{-11}$ M, $5 \times 10^{-11} - 5 \times 10^{-8}$ M and $5 \times 10^{-8} - 1 \times 10^{-6}$ M concentrations of PA (Figure 13). Consequently, Stern-Volmer constants ($K_{SV}$) have been determined for these three different ranges of concentrations by using exponential equation ($I/I_0=Ae^{k[Q]}+B$)43, 50-52, and were respectively found to be $1.20 \times 10^{11}$ M⁻¹, $1.10 \times 10^8$ M⁻¹, $4.8 \times 10^5$ M⁻¹. The lowest limit of detection for PA was $5 \times 10^{-13}$ M.53

Figure 13: Plot of fluorescence intensity ($I/I_0$) of TRIPOD-TP (5μM, H₂O-DMSO; 98:2 vs log[NAC])
In order to rationalize the interaction of TRIPOD-TP with PA, $^1$H NMR spectrum of TRIPOD-TP before and after addition of PA was recorded in DMSO-$d_6$-water (7:3) at 5 mM concentration. It could not be recorded in water (2% DMSO), the solvent of all photophysical and morphological studies, due to its poor solubility in this medium at such high concentration. In $^1$H NMR spectrum of the solution of 1:1 mixture of TRIPOD-TP and PA, the up-field shift of 2H singlet of PA to δ 8.32 from 8.62 (in $^1$H NMR spectrum of PA) points to the encapsulation of PA in the cavity of TRIPOD-TP (Figure 14).

**Figure 14:** Effect of addition of PA on $^1$H NMR spectrum of TRIPOD-TP (5 mM, DMSO + H$_2$O; 7:3)

In order to further explore the utility of TRIPOD-TP for the detection of PA vapors, the thin film was fabricated on glass plate by drop-cast technique [20 μl of TRIPOD-TP (10 μM)]. The fluorescence intensity of the thin film was recorded as it was exposed to the saturated vapors of PA at regular intervals of time. The fluorescence intensity of the thin film started decreasing as soon as it came in contact with PA vapors and initial fluorescence emission intensity significantly decreased to 25% after exposure of the thin film for 120 sec. Upon continuous exposure with PA vapors, within 360 sec the maximum fluorescence quenching was observed (Figure 15). The thin film exposed to saturated vapors of PA for 600 sec on washing with water did not revive the fluorescence intensity. Therefore, binding of PA on thin films is irreversible. The non-variation in life time of thin films in the presence of PA vapors supports static mechanism.

Similarly, the addition of 0.1 equivalent of other NACs like 2,4-DNP, TNT, and Cl-DNB to the solution of TRIPOD-TP demonstrated 70, 39 and 20 % quenching of
fluorescence intensity and exhibited amplified quenching effect at < 0.1 equiv. of TRIPOD-TP. In all these cases, the plot of fluorescence intensity vs [NAC] shows non-linear change (Figure 16a-c) in fluorescence quenching of the solution of TRIPOD-TP. The plot of I/I₀ vs. log [NAC] demonstrates more than one segment for linear change in I/I₀ with respect to log [NAC] (Figure 13). The K_{SV} values for these NACs have been determined using exponential equation (I/I₀ = Ae^{k_{SV}[Q]} + B)\textsuperscript{43, 50-52} and are given in table 1. The minimum detection limits\textsuperscript{53} for 2,4-DNP, TNT and Cl-DNB were 50 x 10^{-12} M, 200 x 10^{-12} M and 1 nM, respectively and were at concentrations higher by > 100 times or more in comparison to the lowest detection limit observed for PA. Therefore, the sensitivity of TRIPOD-TP is in the order PA > 2,4-DNP > TNT > Cl-DNB.

**Table 1:** K_{SV} values for interaction of TRIPOD-TP with NACs by applying exponential equation I/I₀ = Ae^{k_{SV}[Q]} + B from non-linear curve-fitting using Origin software

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<th>NAC</th>
<th>Conc. Range (M)</th>
<th>K_{SV} (M⁻¹)</th>
<th>R value</th>
<th>LOD</th>
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LOD = lower limit of detection; max[x] = after addition of x equiv. of NAC, only a residual change in FI is observed.
Figure 16: Fluorometric titration of TRIPOD-TP (5 µM, H₂O: DMSO, 98:2) with incremental addition of (a) 2,4-DNP, (b) TNT and (c) Cl-DNB, λₑₓ = 290 nm
Figure 15: The variation in fluorescence intensity of drop-casted thin film of TRIPOD-TP on exposure to PA vapors

The cause of selectivity of TRIPOD-TP towards PA over TNT, Cl-DNB and 2,4-DNP could be rationalized based on the energies of HOMO and LUMO of TRIPOD-TP and NACs as calculated from their energy optimized structures. The density functional theory based calculations at B3LYP /6-31G basis set revealed that the picrate anion has its HOMO at ~35.8 kcal/mol above the energy of LUMO of TRIPOD-TP (Figure 17). Therefore, the electron transfer from HOMO of picrate anion to LUMO of TRIPOD-TP results in fluorescence quenching. In case of 2,4-DNP, although its HOMO lies above the LUMO of TRIPOD-TP but due to larger energy difference (~56 kcal/mol) between the HOMO of 2,4-DNP anion and LUMO of TRIPOD-TP, the efficiency of electron transfer and thus efficiency of fluorescence quenching is significantly lowered. The theoretical calculations also revealed that HOMO of TNT and Cl-DNB are lower in energy than LUMO of TRIPOD-TP and is responsible for the poor sensitivity of TRIPOD-TP towards TNT and Cl-DNB. Therefore, TRIPOD-TP undergoes more efficient fluorescence quenching with PA than with 2,4-DNP, TNT and Cl-DNB.
Figure 17: Pictorial representation of the electron transfer phenomenon which occurs from the HOMO of picrate anion to the LUMO of TRIPOD-TP. All theoretical calculations are performed using density functional theory at B3LYP/6-31 G basis set.

3.2.5 Contact mode methods for detection of traces of PA

Finally, we investigated the applicability of TRIPOD-TP in both qualitative and quantitative detection of picric acid using contact mode method. For this, we carried out experiments on paper strips coated with TRIPOD-TP. The paper strips were then visualized under 365 nm UV light. We observed that the addition of 10 μl of 10^{-17} M PA causes visible quenching of the fluorescence intensity on the paper strip (Figure 18B). Complete quenching of fluorescence intensity was observed with 10^{-9} M PA (Figure 18E) and addition of higher concentrations of PA caused black color area developed on paper strip. Figure 18A shows no quenching of fluorescence with only water. Therefore, the minimum amount of PA detectable is 2.29 x 10^{-20} g/cm² under 365 nm light illumination. For 10 μl of 10^{-17} M solution, it amounts to 60 molecules of PA.
Figure 18: Photographs of fluorescence quenching (under 365 nm UV light) of paper strips coated with TRIPOD-TP and different amounts of PA; (A) Paper strip with a drop of water; (B) 10^{-17} M (C) 10^{-15} M (D) 10^{-13} M (E) 10^{-9} M. [The size of each paper strip is 1.0 cm²]

In order for quantification of PA using TRIPOD-TP coated paper strips, the fluorescence spectra of the above paper strips treated with different concentrations of PA were recorded. The plot of fluorescence intensity vs log [PA] shows linear change and is in agreement with exponential decrease in fluorescence intensity (Figure 19) with addition of PA as observed in the solution phase. The $K_{sv}$ value as determined by exponential equation $I/I_0 = A e^{K_{sv}[Q]} + B$ is found to be $2.48 \times 10^{11}$ M⁻¹ and is in close agreement with $K_{sv}$ ($1.20 \times 10^{11}$ M⁻¹) that observed in solution phase at low concentrations of PA. The addition of water did not quench the fluorescence intensity of the paper strip. Therefore, paper strips coated with TRIPOD-TP were highly sensitive towards PA and could be used for quantification of $10^{-17}$ M - $10^{-6}$ M PA solutions.

Figure 19: (a) Front surface steady-state fluorescence quenching of TRIPOD-TP with PA. PS = fluorescence spectrum of TRIPOD-TP coated paper strip; PS + water = spectrum after addition of 200 μl of water to PS. $10^{-17}$-$10^{-6}$ correspond to molar concentrations of PA added to PS. (b) Plot of fluorescence intensity of TRIPOD-TP vs log [PA] M
3.3 CONCLUSION

Thus, we have synthesized a 1-(p-terphenyl)benzimidazolium based probe TRIPOD-TP which depending on the amount of PA could undergo complex aggregation – disaggregation process. This was associated with amplified (> 88%) fluorescence quenching to enable ultra-trace detection of picric acid (PA). FE SEM and TEM studies along with DLS investigations provided deep insight into the aggregation - disaggregation process. In solution phase TRIPOD-TP could detect $5 \times 10^{-13}$ M PA. Paper strips coated with TRIPOD-TP could detect as low as 10 μl of $10^{-17}$ M PA both qualitatively and quantitatively using naked eye (under 365 nm lamp) visible fluorescence quenching and on measuring front surface steady state fluorescence, respectively. The other NACs viz. 2,4-DNP, TNT, Cl-DNB also caused amplified fluorescence quenching but at significantly higher concentrations. Therefore, the present study provides a new insight into the design of self-assembled molecular probes for the detection of nitro-aromatic explosives.

3.4 Experimental Section

3.4.1 Materials and Reagents: All chemicals were obtained from common suppliers (Aldrich, SD fine chemicals limited, Spectrochem etc.) and were used without further purification. The deionized water was obtained from ULTRA UV/UF Rions Lab Water System Ultra 370 series.

3.4.2 Instrumentation: $^1$H and $^{13}$C NMR spectra were recorded on BRUKER Bio spin AVANCE-III FT NMR HD-500 spectrophotometers using CDCl$_3$ or DMSO-$d_6$ as solvent and tetramethylsilane (TMS) as internal standard. Data are reported as follows: chemical shifts in ppm, coupling constants J in Hz; multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet). High resolution mass spectra were recorded on BRUKER DALTONIK micrOTOF-Q11 spectrometer. UV-Vis studies of compounds were performed on SHIMADZU-2450 spectrophotometer, with a quartz cuvette of path length 1 cm. The cell holder was thermo stated at 25.0 ± 0.2°C. The fluorescence spectra were recorded on BH-CHRONOS fluorescence spectrophotometers with a quartz cuvette of path length 1 cm. The cell holder was thermo stated at 25.0 ± 0.2°C. The scanning electron microscope
(SEM) images were obtained with a field emission scanning electron microscope SEM JEOL JSM-6610LV. The transmission electron microscope (TEM) images were obtained with JEOL JEM-2100 Electron Microscope. DLS experiments were performed on Malvern-Zetasizer. The time resolved fluorescence spectra were recorded with BH-CHRONOS time-resolved fluorescence spectrophotometer.

3.4.3 Quantum yield calculations: Fluorescence quantum yields (Φs) were determined by using an optically matching solution of Quin-hydrogen sulphate (Φs = 0.54 in 0.1 M H2SO4) as standard at excitation wavelength of 290 nm and quantum yield is calculated using equation

$$\Phi_s = \Phi_r \frac{A_r}{A_s} \frac{n_s^2}{n_r^2} \frac{D_s}{D_r}$$

Φs and Φr are the radiative quantum yields of sample and the reference, respectively. Ds and Dr are the respective areas of emission for the sample and reference, respectively. As and Ar are the absorbance; ns and nr are the refractive indices of the sample and reference solutions, respectively.

3.4.4 UV-Vis and Fluorescence Titrations: Stock solution of TRIPOD-TP (1 mM) was prepared in DMSO. For experiments with TRIPOD-TP, we have taken 3 ml of the solution that contains 15 µL solution of TRIPOD-TP in DMSO, 60 µL DMSO and 2.94 ml water in cuvette. Typically, aliquots of freshly prepared standard solutions (10⁻¹ M) of sodium salts (Na⁺X⁻), where X = CN⁻, F⁻, Cl⁻, Br⁻, I⁻, ClO₄⁻, NO₃⁻, SO₄²⁻, HSO₄⁻, SCN⁻, AcO⁻ and H₂PO₄⁻ in deionized millipore water were used to record UV-Vis and fluorescence spectra. The standard solutions (10⁻¹ M) of NACs, phenol, 2-nitrophenol (2-NP), 4-nitrophenol (4-NP), 2,4-dinitrophenol (2,4-DNP), picric acid (PA), 4-hydroxybiphenyl (4-OHBP); 2-nitrotoluene (2-NT), 2,4-dinitrotoluene (2,4-DNT), dinitrobenzene (DNB), 2-chloronitrobenzene (2-CINB), trinitrotoluene (TNT); 3-chloronitrobenzene (3-CINB), 4-chloro-nitrobenzene (4-CINB), chloro-2,4-dinitrobenzene (Cl-DNB); 2-nitroaniline (2-NA) and 2,4-dinitroaniline (2,4-DNA) were prepared in DMSO.
3.4.5 Detection limit: The detection limit was calculated based on the fluorescence titration. To determine the S/N ratio, the emission intensity of TRIPOD-TP (5 μM) without picric acid was measured by 5 times and the standard deviation of blank measurements was determined. Under the present conditions, a good linear relationship between the fluorescence intensity and the picric acid concentration could be obtained between $5 \times 10^{-13} - 5 \times 10^{-11}$ M ($R = 0.991$), $5 \times 10^{-11} - 5 \times 10^{-8}$ M ($R = 0.981$) and $5 \times 10^{-8} - 1 \times 10^{-6}$ M ($R = 0.997$) concentrations of PA for TRIPOD-TP. The detection limit is then calculated with the equation:

$$\text{Detection limit} = 3\sigma_{bi}/m$$

Where, $\sigma_{bi}$ is the standard deviation of blank measurements; $m$ is the slope between intensity versus sample concentration. The detection limit was measured to be $5 \times 10^{-13}$ M at S/N = 3.

3.4.6 Synthesis of TRIPOD-TP

Synthesis of compound 1: $p$-Bromoterphenyl (4.3 g, 14 mmol), CuI (267 mg, 1.4 mmol) and benzotriazole (333 mg, 2.8 mmol) were dissolved in DMSO (10 ml). To this stirred mixture benzimidazole (4.2 g, 35 mmol) and K-OtBu (4.3 g, 38.5 mmol) were added under N$_2$ and resulting solution was heated at 120ºC for 24h. The reaction mixture was cooled to room temperature and aqueous solution of EDTA (2.8 mmol) was added. The compound was extracted with ethyl acetate (3 × 30 ml). The solvent was distilled off, and residue on column chromatography with hexane-chloroform (9:1) mixture as eluent gave pure compound 1, white solid (2.9 g). Yield 60%; m. p. 192 ºC. $^1$H NMR (300 MHz, CDCl$_3$) : $\delta$ 7.35 - 7.41 (m, 3H, ArH), 7.49 (t, 2H, $J = 7.35$ Hz, ArH), 7.61 - 7.69 (m, 5H, ArH), 7.74 (s, 4H, ArH), 7.85 (d, 2H, $J = 8.4$ Hz, ArH), 7.90 - 7.93 (m, 1H, ArH), 8.18 (s, 1H, Bim C2-H). $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ 110.6, 120.7, 123.0, 123.8, 124.4, 127.1, 127.4, 127.5, 127.6, 127.6, 127.8, 128.6, 128.6, 128.9, 132.0, 133.8, 135.5, 138.6, 140.5, 140.6, 140.8, 142.3, 144.2. HRMS-ESI : calcd for C$_{25}$H$_{18}$N$_2$, m/z = 347.1504 [M]; found, 347.1518 [M].
Synthesis of TRIPOD-TP: The solution of 1-(p-terphenyl)benzimidazole (260 mg, 0.75 mmol) and 1,3,5-tris(bromo-methyl)-2,4,6-triethylbenzene (110 mg, 0.25 mmol) was heated in acetonitrile (15 ml) at 90°C under N₂ for 24 h. The solid separated was filtered and washed with acetonitrile to get white solid of TRIPOD-TP (335 mg); yield 90.5 %; m. p. 245°C; ¹H NMR (500 MHz, DMSO-d₆): δ 1.26 (bs, 9H, 3 × CH₃), 2.50 (bs, 6H, 3 × CH₂), 5.88 (s, 6H, 3 × CH₂), 7.34-7.40 (m, 9H, ArH), 7.52 (d, 6H, J = 6.5 Hz, ArH), 7.64 (s, 12H, ArH), 7.82 (t, 3H, J = 7.75 Hz, ArH), 7.90-7.94 (m, 18H, ArH), 8.56 (d, 3H, J = 8.5 Hz, ArH), 10.0 (s, 3H, Bim C2-H). ¹³C NMR (125 MHz, DMSO-d₆): δ 16.0, 24.3, 45.7, 114.2, 114.7, 118.6, 126.8, 127.1, 127.7, 127.8, 128.1, 128.2, 128.4, 129.5, 132.0, 132.2, 132.6, 137.6, 139.3, 140.2, 141.7, 141.8, 149.0. HRMS-ESI: calcd for C₉₀H₇₅Br₃N₆, m/z = 659.2618, 660.2613 [M-2Br]²⁺, 413.2012 [M-3Br]³⁺; found, 659.2890, 660.2897 [M-2Br]²⁺, 413.2190 [M-3Br]³⁺.
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