Supporting Information

Dual "Static and Dynamic" Fluorescence Quenching Mechanisms Based Detection of TNT via a Cationic Conjugated Polymer

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Table of Contents

Caution! Explosive materials such as TNT and PA, possess high explosive nature, and should be used carefully in low amount with appropriate safety measurements.

Materials and Methods. All the chemicals were purchased commercially from various chemical companies. TNT was synthesized by previous established method.(Ref. 27 manuscript) PA was purchased from Loba Chemie Pvt. Ltd. Other NACs such as 2,6-dinitrotoluene (2,6-DNT), 1,3-dinitrobenzene (1,3-DNB), 4-nitrotoluene (4-NT), and 2,4-dinitrotoluene (2,4-DNT) and sodium hydroxide (NaOH) were purchased from Sigma-Aldrich Chemicals and used as received. All other chemicals were purchased from Merck or Alfa-Aesar. MiliQ water was used in all the sensing experiments. PFPy stock solution (1×10^{-3} M) was prepared in DMSO solvent. Stock solutions of most of NACs (1×10^{-3} M) were prepared in methanol or water depending on the solubility. Concentration of PFPy was fixed throughout the sensing experiment i.e. (6.66×10^{-6} M). All NMR spectra were recorded on Bruker Ascend 600 spectrometer. Fluorescence and absorption spectra were recorded on Horiba Fluoromax-4 spectrofluorometer and Perkin Elmer Lambda-25 spectrophotometer, respectively using 1 cm × 1 cm dimension of quartz cuvettes at room temperature. Time-resolved photoluminescence (TRPL) spectra were recorded using Edinburg Life Spec II instrument. Gel Permeable Chromatography (GPC) was performed in chloroform using polystyrene as standard. Test strips studies were performed by using Whatman paper (grade I). All the experimental data were collected after 2 minutes interval.

Synthesis and characterisation of PFPy. The synthesis of the desired copolymer PFPy is depicted in Scheme 1. The products (monomer and polymer) at each step were purified and well characterized (Figures S1-S5). PF acts as a precursor polymer for PFPy synthesis. Cationic pyridinium units, that work as key receptor sites for the analyte TNT binding, also makes it soluble in polar solvents (water, DMSO, etc.), were linked to the pendant chains to generate the final copolymer PFPy. The aqueous solution of PFPy displayed UV-vis absorption peak at 430 nm and emission peak at 553 nm (excitation wavelength = 430 nm) (Figure S6) with a remarkable fluorescence quantum yield (Φ_s) of 0.59 in water. The 553 nm fluorescence emission peak obtained for PFPy at pH=12 was chosen as a medium for TNT sensing (Figure S7).

Synthesis of M2. Monomer (M2) was synthesized by previously established procedure. (Ref. 22 manuscript) A mixture of fluorene (500 mg, 3.008 mmol), tertrabutylammonium iodide (TBAI) (0.238 g, 0.6015 mmol) and 50 % NaOH solution (w/v) were taken in 50 mL round bottom flask (RBF) under inert atmosphere and Freeze-thaw cycles alternatively. Then 1,6-dibromohexane (3.24 mL, 21.05 mmol) was added to the reaction mixture. RBF was maintained at 70 °C and continuously stirred for 4 h. After that, work up was done and the organic layer was extracted using dichloromethane (DCM) and washed with water thrice. Organic layer was dried over anh. sodium sulfate, crude product was collected after evaporation of DCM, which was purified via column chromatography (yield ~80 %). ^{1}H NMR (400MHz, $CDCl_3$, δ): 7.52 (d, 2H), 7.46 (dd, 2H), 7.43 (d, 2H), 3.30 (t, 4H), 1.91 (m, 4H), 1.21 (m, 4H), 1.18 (m, 4H), 1.07 (m, 4H), 0.58 (m, 4H). ^{13}C NMR (150MHz, $CDCl_3$, δ): 152.36, 139.27, 130.54, 126.28, 121.78, 121.46, 55.77, 40.28, 34.16, 32.83, 29.18, 27.98, 23.67.

Synthesis of PF: A mixture of M2 (200 mg, 0.31 mmol), 2,1,3-Benzothiadiazole-4,7-bis(boronic acid pinacol ester) (120 mg, 0.31 mmol), tetrakistriphenylphosphine palladium (18 mg, 0.016 mmol), potassium carbonate (0.428 mg, 3.1 mmol) pre-dissolved in water (3 mL), and then THF (9 mL) was added to Schlenk flask under inert atmosphere and subjected to three freeze-thaw cycles. The reaction mixture was maintained at 80 °C for 18 h. Further, the end capping was done via addition of phenyl iodide followed by addition of benzene boronic acid. After that, it was concentrated at reduced pressure then extracted with DCM, which was then washed with water thrice and poured into methanol to obtain precipitates. These precipitates were redissolved in DCM and reprecipated in methanol thrice, to get yellowish orange solid product. (Yield = 77 %) ^{1}H NMR (400MHz, $CDCl_3$, δ): 8.02 (b), 7.96 (b), 7.71

(b), 7.50 (b), 7.38 (b), 3.29 (b), 2.08 (b), 1.67 (b), 1.25 (b), 1.13 (b), 0.76 (b). GPC using polystyrene as standard in Chloroform: $M_w = 1.08 \times 10^4$, PDI= 2.6.

Synthesis of PFPy: In a 10 mL RBF, PF (60 mg, 0.095 mmol) was dissolved in DMF and then pyridine (0.077 mL, 0.958 mmol) was added to it. The flask was maintained at 70 °C under continuous stirring for 24 h. After that, the reaction mixture was first poured in diethyl ether to get precipitates followed by precipitation in DCM thrice. The precipitates were dried under vacuum to get yellowish orange polymer (PFPy). (Yield = 80%) ^{1}H NMR (600MHz, DMSO-d6, δ): 8.97 (b), 8.55 (b), 8.07 (b), 7.88 (b), 7.75 (b), 7.56 (b), 7.50 (b), 7.37 (b), 4.46 (b), 2.08 (b), 1.71 (b), 1.09 (b), 1.07 (b), 0.62 (b).

TRPL Measurements. TRPL measurements of PFPy (6.66×10^{-6} M) were carried out in the absence and presence of various concentration of TNT using pulse excitation of 445 nm and emission at 550 nm in water containing 10 mM NaOH. (Table S1).

Test Strips Preparation. Whatman (grade I) filter paper was first dipped in the solution of PFPy (6.66×10^{-6} M) in DMSO and then in water containing 10 mM NaOH. Test strips were dried to achieve portable fluorescent strips. These strips were cut in desired shapes and sizes (1 cm × 1 cm) and further used directly for portable sensing.

Fluorescence quantum yield (Φ_s). Φ_s was obtained by means of absolute fluorescent quantum yield via the integrating sphere method using a Horiba Fluoromax4 fluorescence spectrometer.

Limit of detection (LOD). For calculating LOD, various samples of PFPy (6.66×10^{-6} M) in water containing 10 mM NaOH, each having different concentration of TNT (0.0 fm, 3.3 fm, 6.6 fm, 10.0 fm, 13.3 fm) were taken and fluorescence spectra were recorded at a fixed excitation wavelength of 430 nm. A calibration plot between PL intensity maxima and TNT concentration resulted in a regression equation. Using the slope (k) of this equation, LOD was calculated via 3σ/k, where σ represents standard deviation in the PL intensity maxima of PFPy in the absence of TNT and k represents slope of the LOD curve.

RET parameters. There are three well know parameters for RET. (1) Overlap integral (J(λ)), (2) Förster distance (R_0) and (3) RET efficiency (E). All the formulae and equations used to determine their value has been mentioned below as well as in the figure (2.3b).[29]

$$J(\lambda) = \int_0^\infty F_D(\lambda)\,\varepsilon_A(\lambda)\lambda^4 d\lambda$$

Where, J(λ) denotes the overlap integral, ε_A denotes molar extinction coefficient of acceptor at wavelength (λ) in $M^{-1}cm^{-1}$, and F_D (λ) denotes corrected fluorescence intensity of PFPy from λ to Δλ with total intensity normalized to unity.

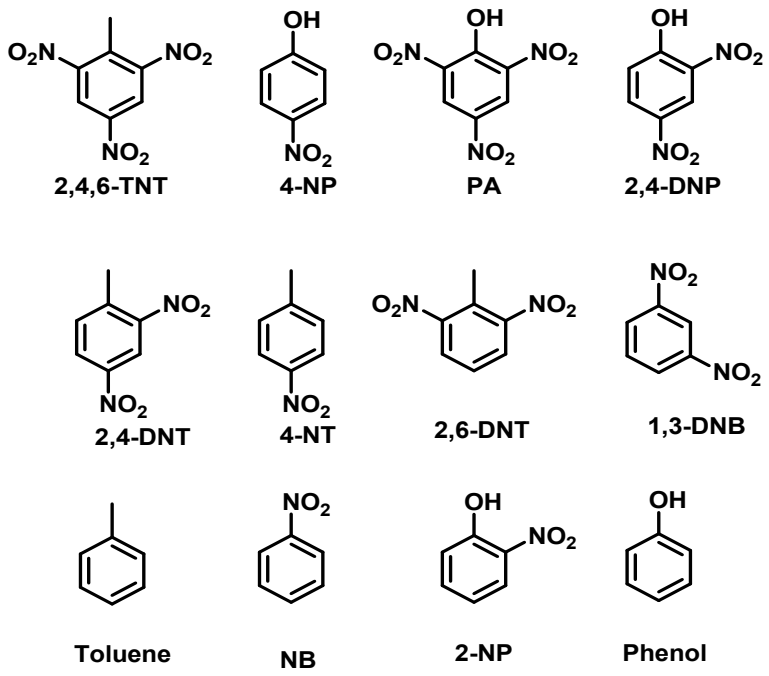
$$R_0 = 0.211[(J)Q(\eta^{-4})(k^2)]^{1/6}$$

Also, R_0 was evaluated via above equation. Where, η denotes refractive index of the medium, Q denotes the fluoresence quantum yield of PFPy , k^2 denotes dipole orientation factor (~ 0.667).

Corrections for Inner filter effect. IFE corrections were performed by using equation mentioned below.[26]

$$I_{corr}/I_{obs} = 10^{(Aex+Aem)/2}$$

Where, I_{corr} denotes the corrected PL intensity and I_{obs} denotes the observed PL intensity. A_{em} denotes absorbance of the solution at excitation wavelength and A_{ex} denotes absorbance of the solution at emission wavelength.



Scheme S1. Structures of various nitroexplosives and electron deficient compounds used in the experiments.

Table S1. : Fluorescence lifetime decay of each component and their fractions in water containing 10 mM NaOH solution.

Sample	τ_1 (ns)	%	τ_2 (ns)	%	χ^2	τ_{avg} (ns)
PFPy	0.764	45.99	3.562	54.00	1.069	2.27
PFPy + TNT (1.0 μM)	0.684	49.91	3.039	50.08	1.016	1.86
PFPy + TNT (2.0 μM)	0.560	51.09	2.520	48.90	1.073	1.51
PFPy + TNT (3.0 μM)	0.529	50.69	2.424	48.31	1.072	1.46

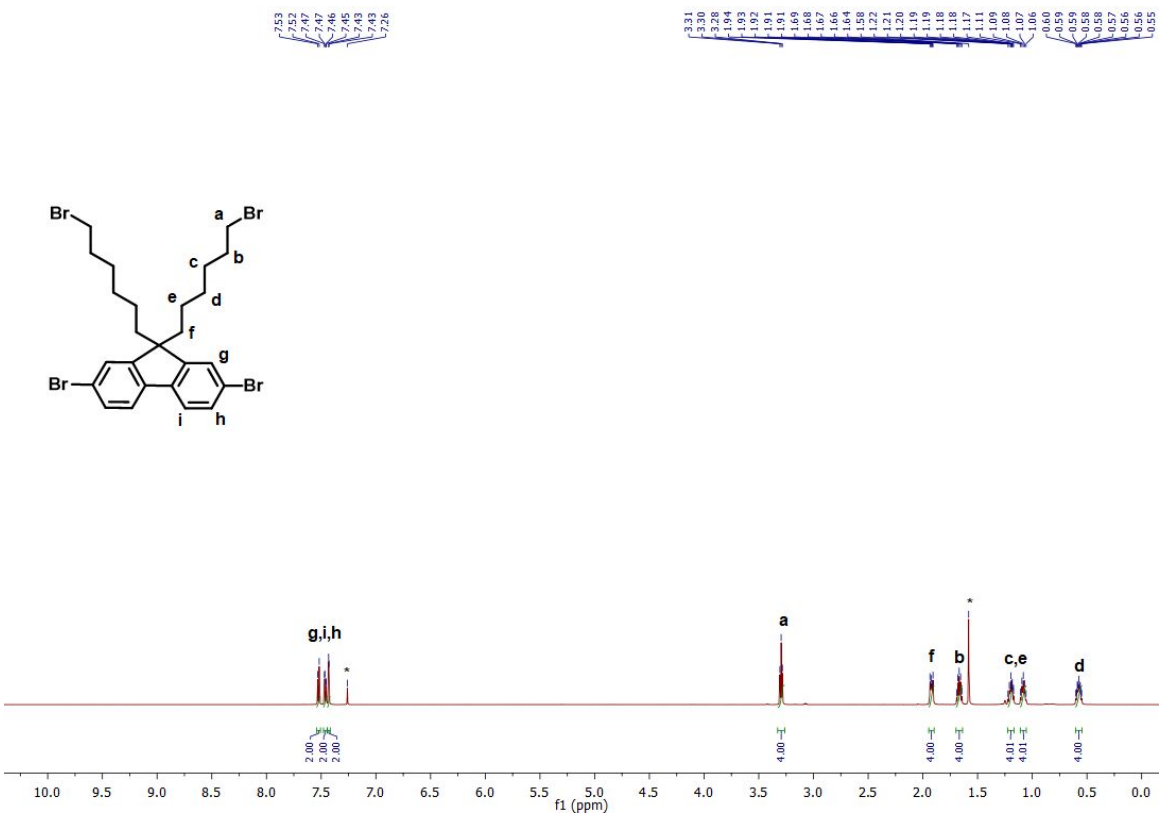


Figure S1. [1]H NMR spectra of M2.

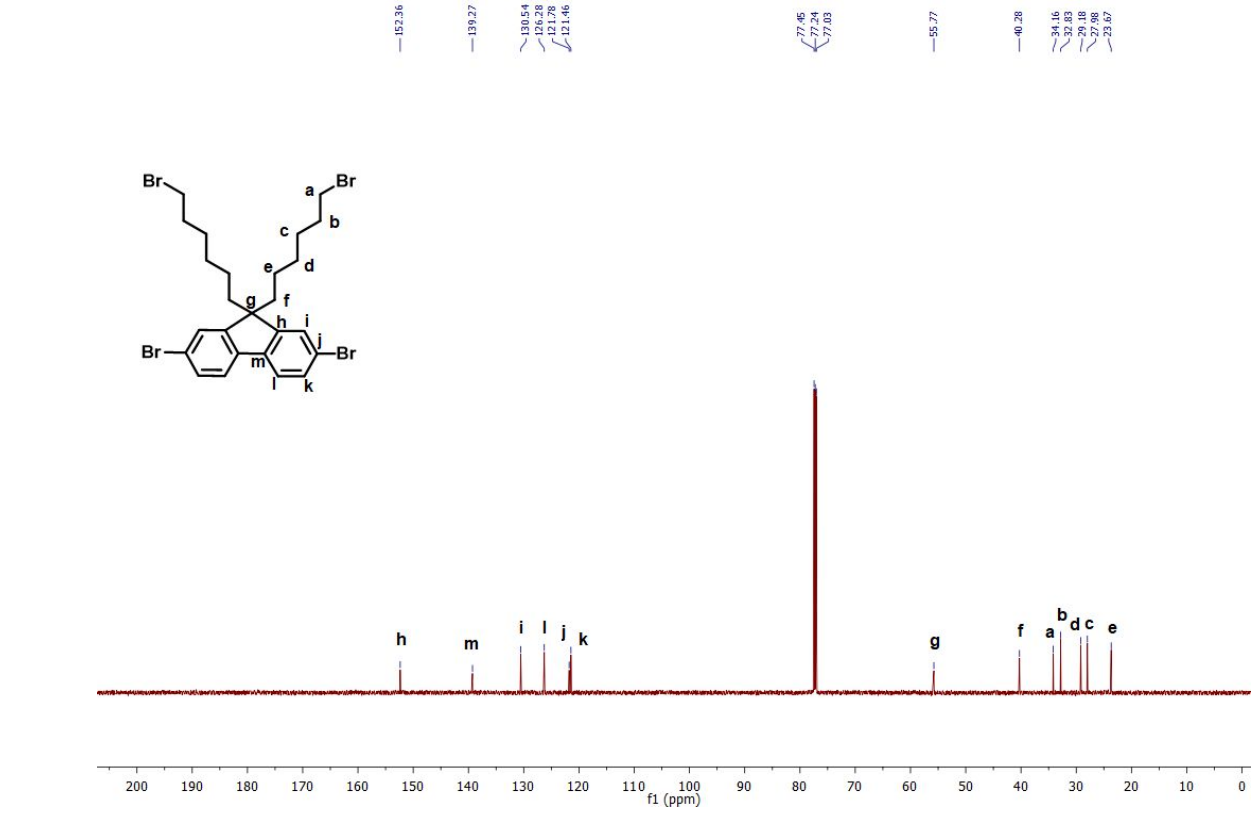


Figure S2. [13]C NMR spectra of M2.

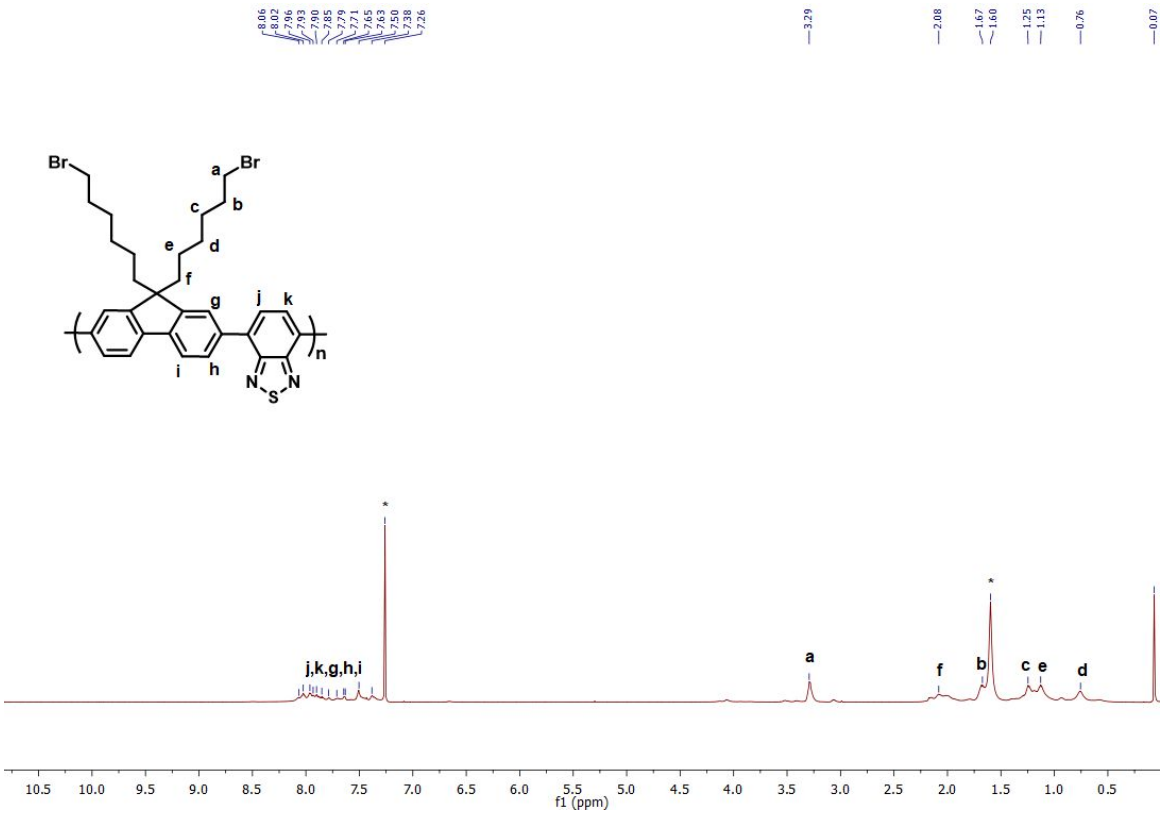


Figure S3. [1]H NMR spectra of PF.

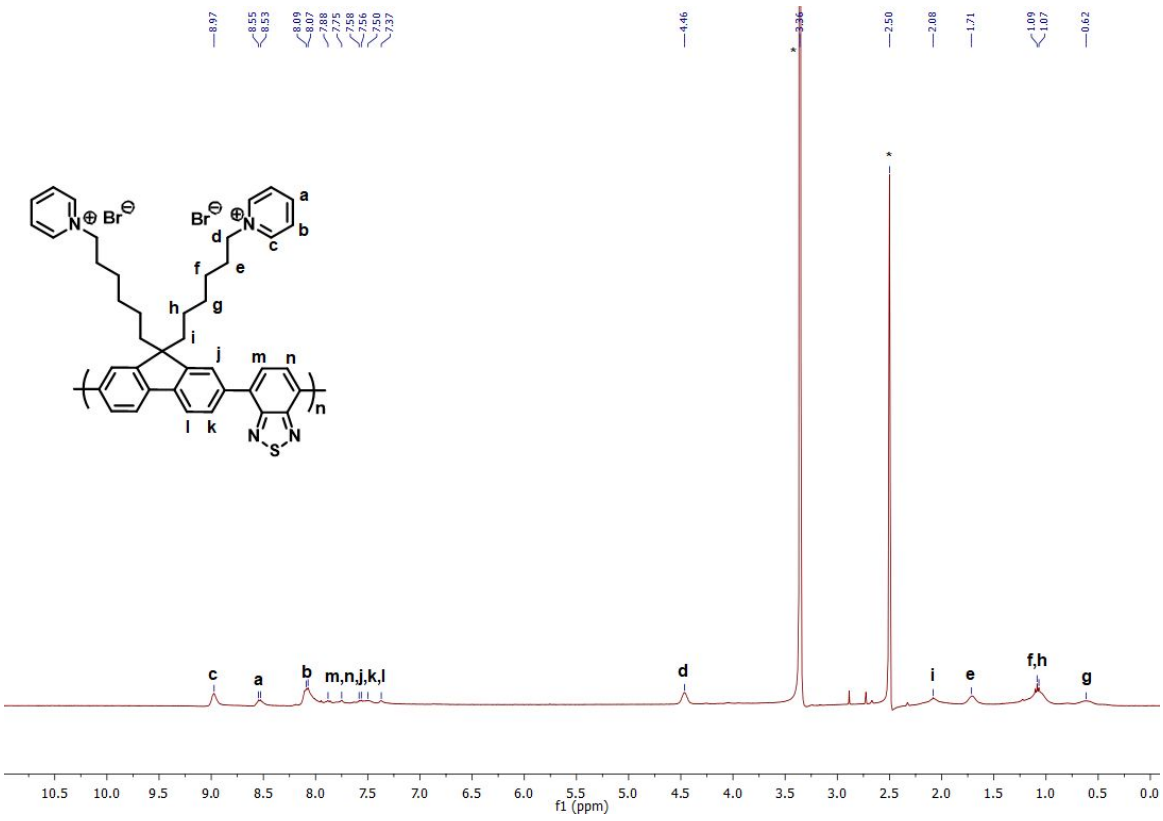


Figure S4. [1]H NMR spectra of PFPy.

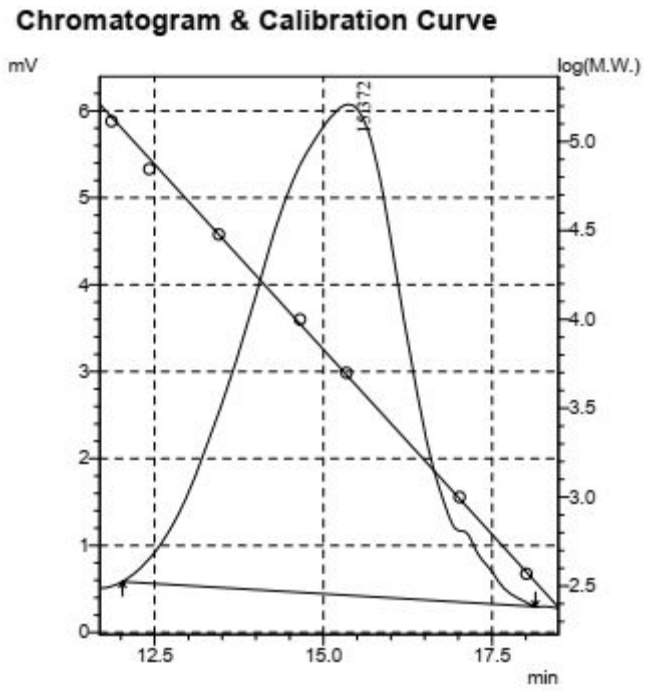


Figure S5. Gel Permeation Chromatogram of polymer-PF.

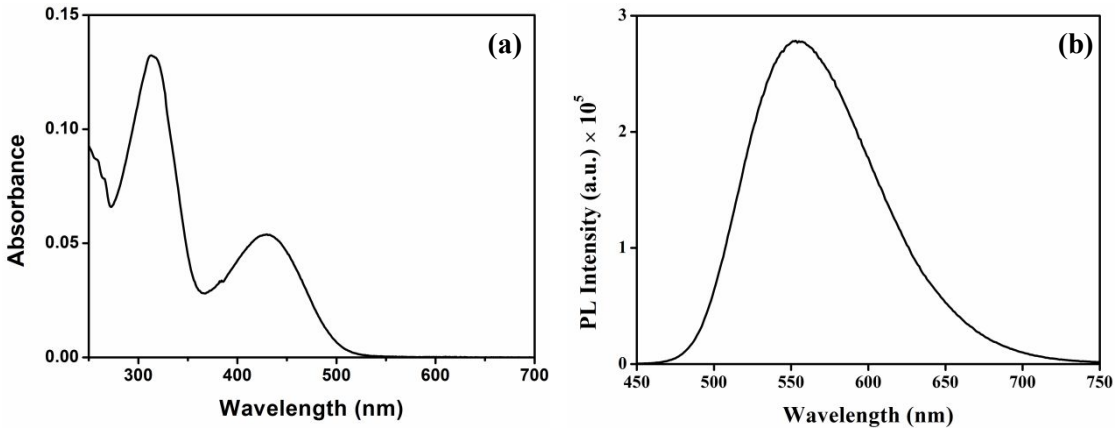


Figure S6. (a) UV-Vis and (b) PL spectrum of PFPy in water.

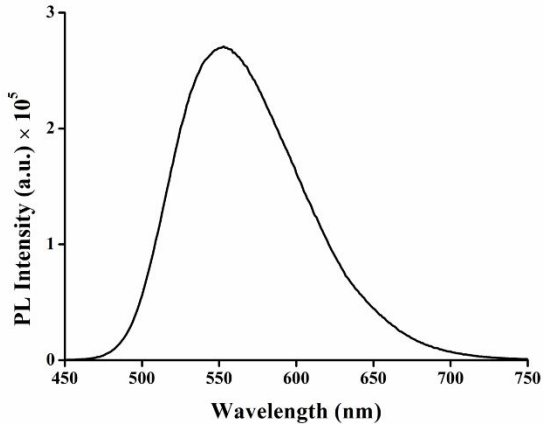


Figure S7. PL spectrum of PFPy in water containing 10 mM NaOH.

Figure S8. Solution of PFPy (6.66×10^{-6} M) in water containing 10 mM–NaOH before and after addition of TNT (3.33×10^{-6} M) under UV lamp (365 nm).

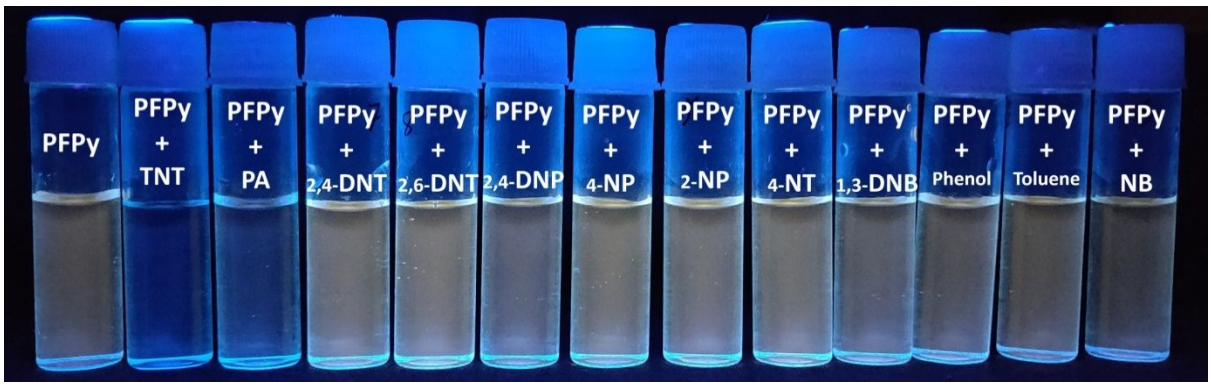


Figure S9. Solution of PFPy (6.66×10^{-6} M) in presence of various nitroexplosive (3.33×10^{-6} M) in water containing 10 mM-NaOH under UV lamp (365 nm).

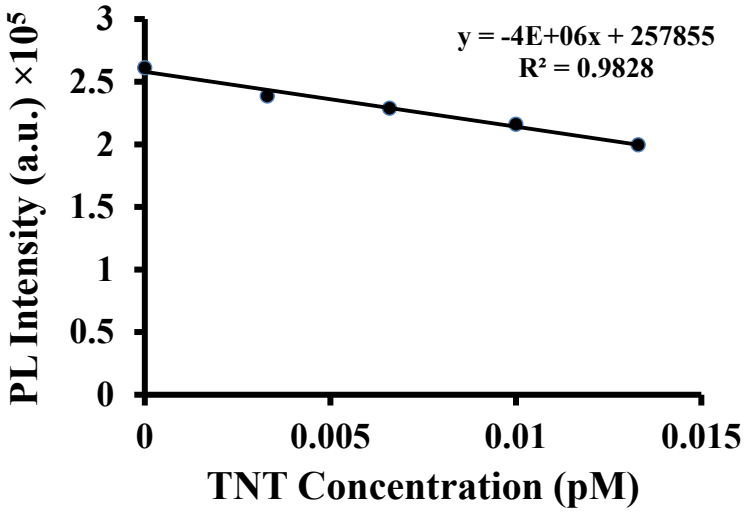


Figure S10. Fluorescence intensity of PFPy *vs* TNT concentration.

LOD = 3 × S.D./k
LOD = 3 × 6592.87 / (4×10^{18})
LOD = 4.94 fM

Table S2: A comparative study of few sensing probes developed for detection of TNT.

Publication	Material Used	Detection Limit	Sensing Mechanism	Solvent	Quenching Constant (M^{-1})
Present Manuscript	Conjugated ionic Polymer (PFPy)	4.94×10^{-15} M (1.12 ppt)	Static and Dynamic Quenching	Water (10 mM NaOH)	$K_S=2.6 \times 10^6$ And $K_D=2.0 \times 10^5$
ACS Appl. Nano Mater. **2019**, 2, 3453-3458	Pyrene-based fluorescent probe	5.0×10^{-9} M	Non-fluorescent complex and charge-transfer interactions	Phosphate buffer (1mM, pH 5.0, containing 1% DMSO (v/v))	6.87×10^5
ACS Appl. Mater. Interfaces **2018**, 10, 27260−27268	Copolymer PFTPBZ	53.7×10^{-6} M and 14.2×10^{-7} M	FRET and PET	Water fractions (95% amd 40 %) in THF	4.03×10^3 and 7.01×10^3
Anal. Chem. **2018**, 90, 3942−3949	MoS_2 quantum dots	1×10^{-9} M	Electron transfer	PBS (0.01 M, pH=7.0)	-
Sensors and Actuators B **2018**, 255, 2628–2634	Copolymer	10×10^{-6} M	Electron transfer	Chloroform	1.3×10^4
Mater. Chem. Front., **2017**, 1, 1875-1880	Hyperbranched CP nanoparticle	3.7×10^{-9} M (0.8 ppb)	-	Water	1.21×10^6
Sensors and Actuators B **2017**, 243, 1002–1009	Carbon Quantum dots	2.13×10^{-7} M	PET	Water	3.9×10^6
Sensors and Actuators B **2017**, 252, 901–911	poly(arylene-ethynylene)s	4.3×10^{-6} M	-	-	4.1×10^4
ACS Appl. Mater. Interfaces **2016**, 8, 8184−8191	MoO_x Quantum dots	1.2×10^{-7} M	IFE	Water (pH=13)	-
ACS Appl. Mater. Interfaces **2016**, 8, 24901−24908	NMPPY−SDBS	100×10^{-9} M	Static Quenching	Water	5.9×10^4
ACS Appl. Mater. Interfaces **2015**, 7, 11956−11964	MOF	4.6×10^{-7} M	Ground state complex	Water (20 mM HEPES; pH=7.0)	3.5×10^4
Anal. Chem. **2015**, 87, 2033−2036	N-rich carbon nanodots	1×10^{-9} M	Charge transfer	Water	-
ACS Appl. Mater. Interfaces **2015**, 7, 21038−21046	Polystyrene derivative	5×10^{-9} M	Electro-static complex	H_2O/ACN (1:1)	-
ACS Appl. Mater. Interfaces **2013**, 5, 8146−8154	Quantum Dots	2.8×10^{-7} M	Electron transfer	Ethanol	-
Chem. Commun., **2012**, 48, 4633–4635	PPV@MSN-NH_2	6×10^{-7} M	FRET	Ethanol	7.42×10^4
Macromolecules **2011**, 44, 5089–5092	Conjugated polymer	23 ppb	PET	Water	1.2×10^5

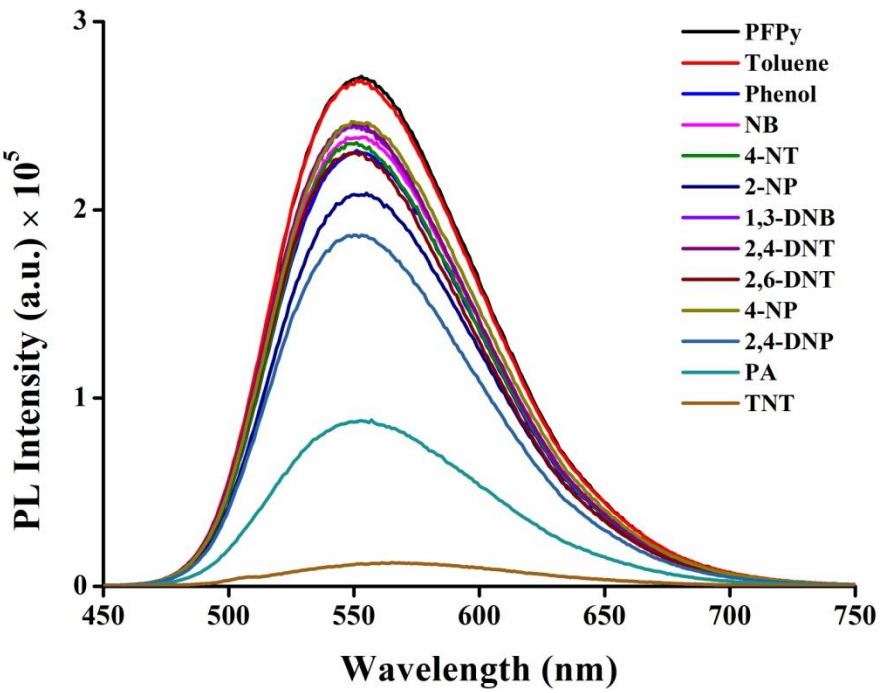


Figure S11. Photoluminescence spectra of PFPy (6.66×10^{-6} M) in presence of various nitroexplosive compounds (3.33×10^{-6} M) in water containing 10 mM-NaOH.

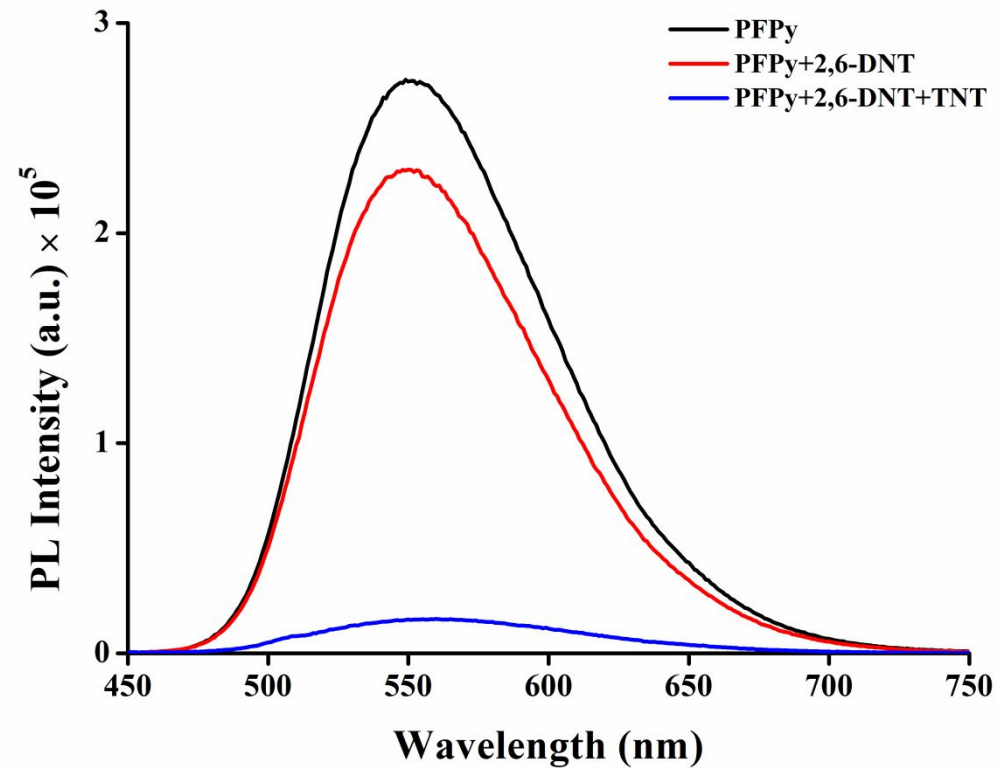


Figure S12. Emission spectra of PFPy (6.66×10^{-6} M) with 2,6-DNT (3.33×10^{-6} M) followed by addition of TNT (3.33×10^{-6} M) in water containing 10 mM-NaOH.

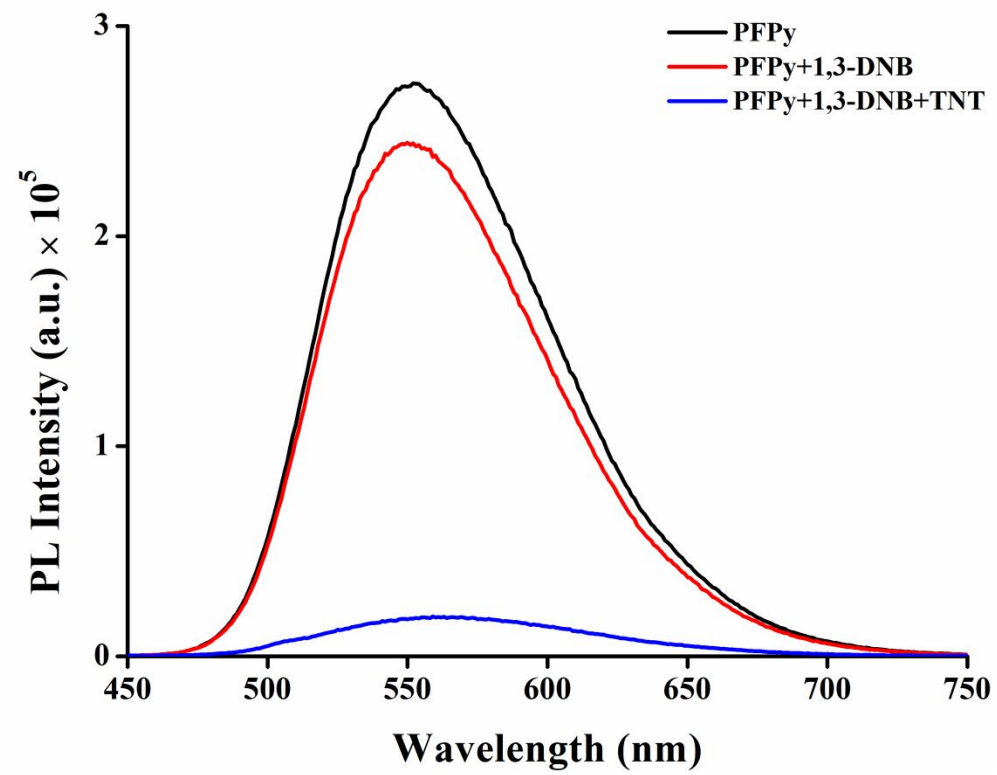


Figure S13. Emission spectra of PFPy (6.66×10^{-6} M) with 1,3-DNB (3.33×10^{-6} M) followed by addition of TNT (3.33×10^{-6} M) in water containing 10 mM-NaOH.

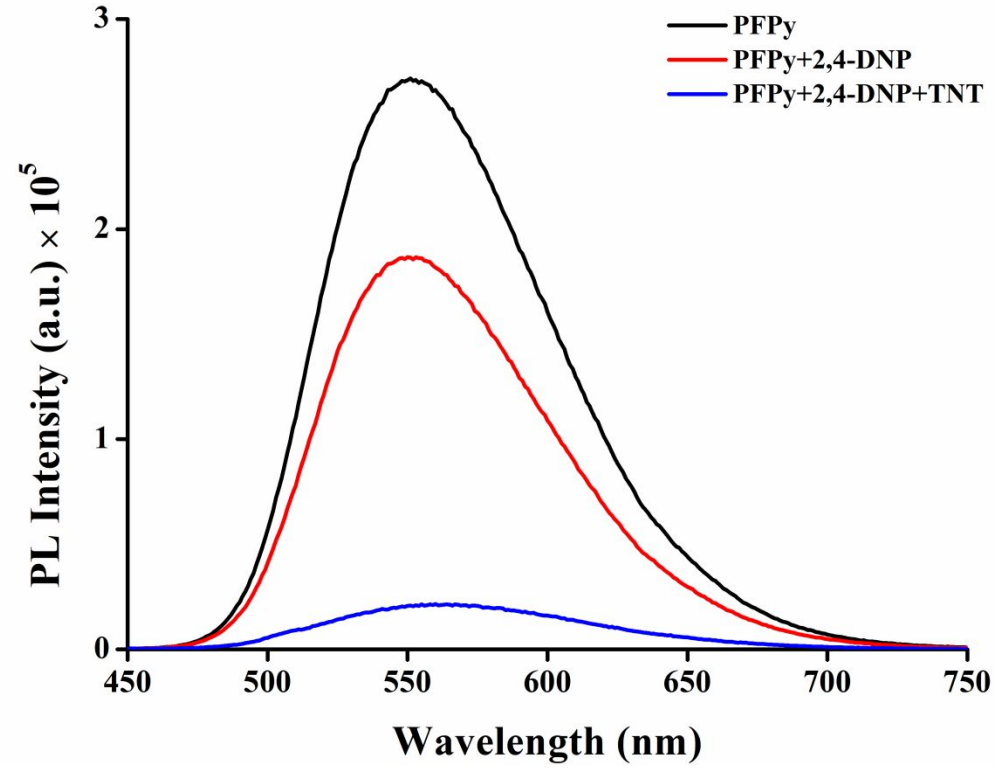


Figure S14. Emission spectra of PFPy (6.66×10^{-6} M) with 2,4-DNP (3.33×10^{-6} M) followed by addition of TNT (3.33×10^{-6} M) in water containing 10 mM-NaOH.

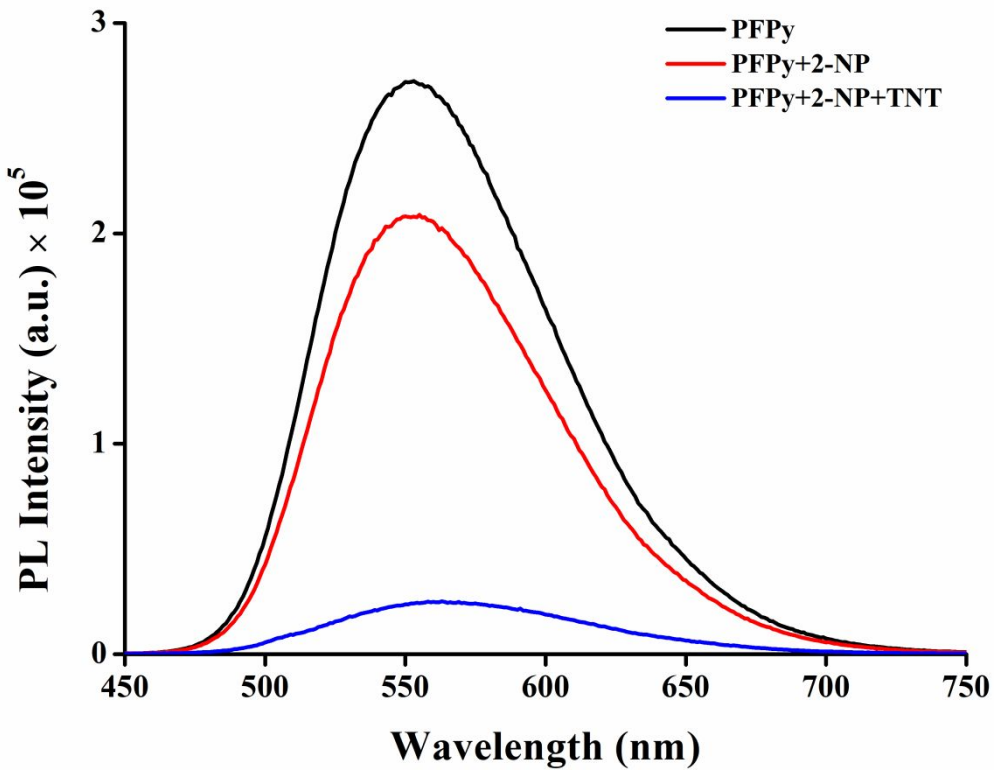


Figure S15. Emission spectra of PFPy (6.66×10^{-6} M) with 2-NP (3.33×10^{-6} M) followed by addition of TNT (3.33×10^{-6} M) in water containing 10 mM-NaOH.

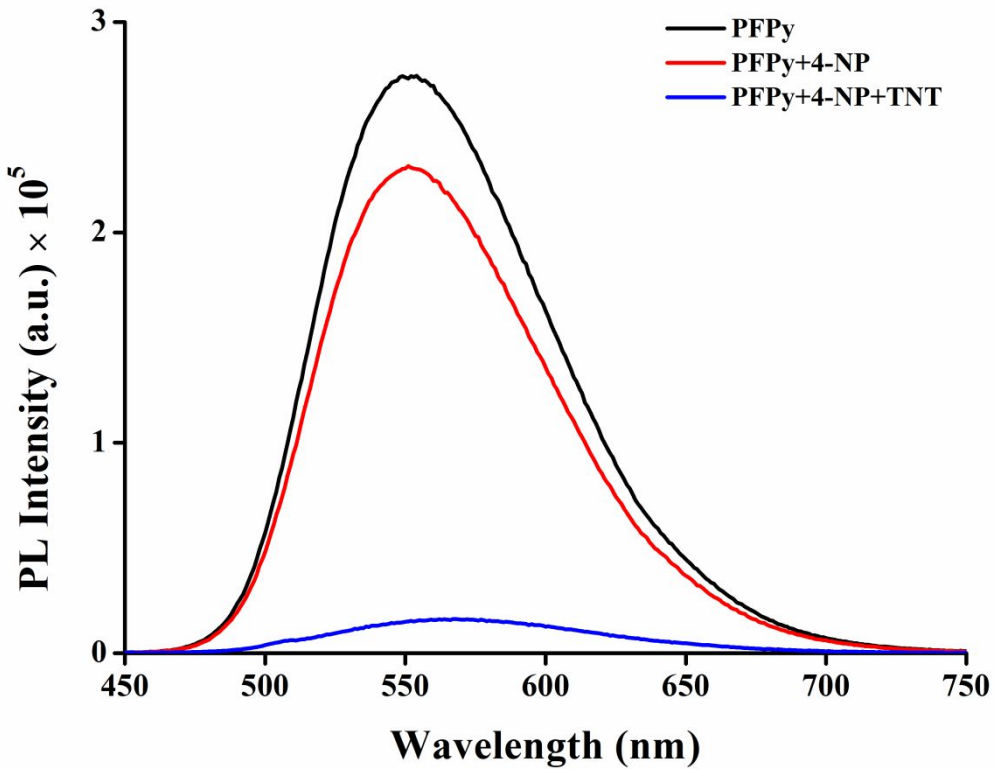


Figure S16. Emission spectra of PFPy (6.66×10^{-6} M) with 4-NP (3.33×10^{-6} M) followed by addition of TNT (3.33×10^{-6} M) in water containing 10 mM-NaOH.

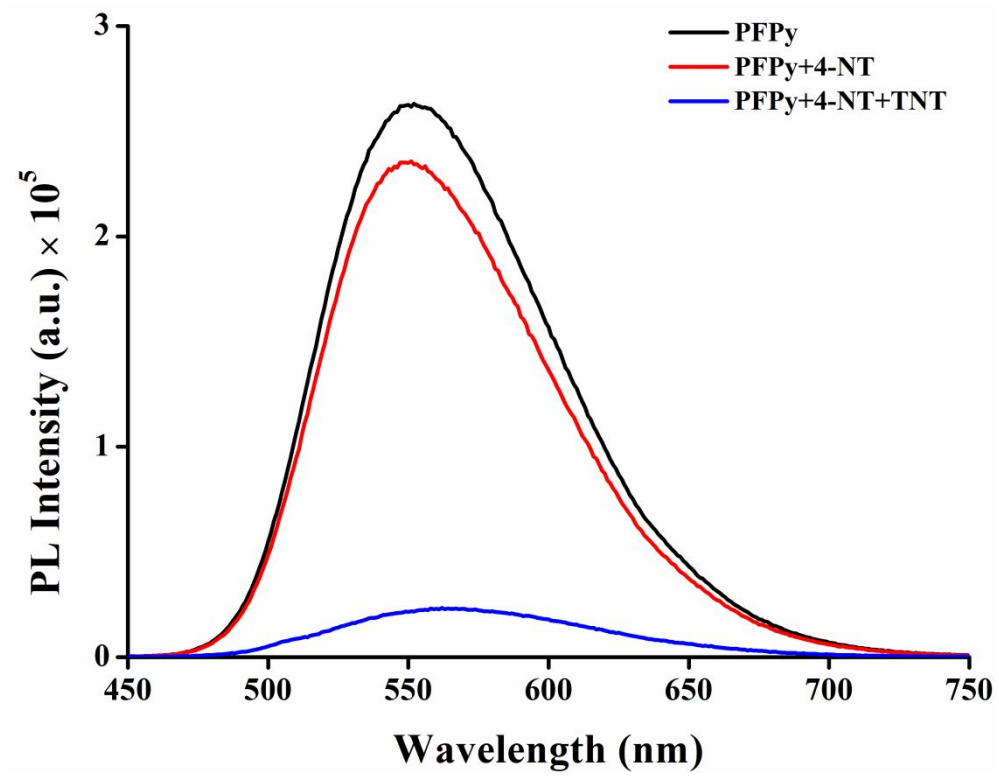


Figure S17. Emission spectra of PFPy (6.66×10^{-6} M) with 4-NT (3.33×10^{-6} M) followed by addition of TNT (3.33×10^{-6} M) in water containing 10 mM-NaOH.

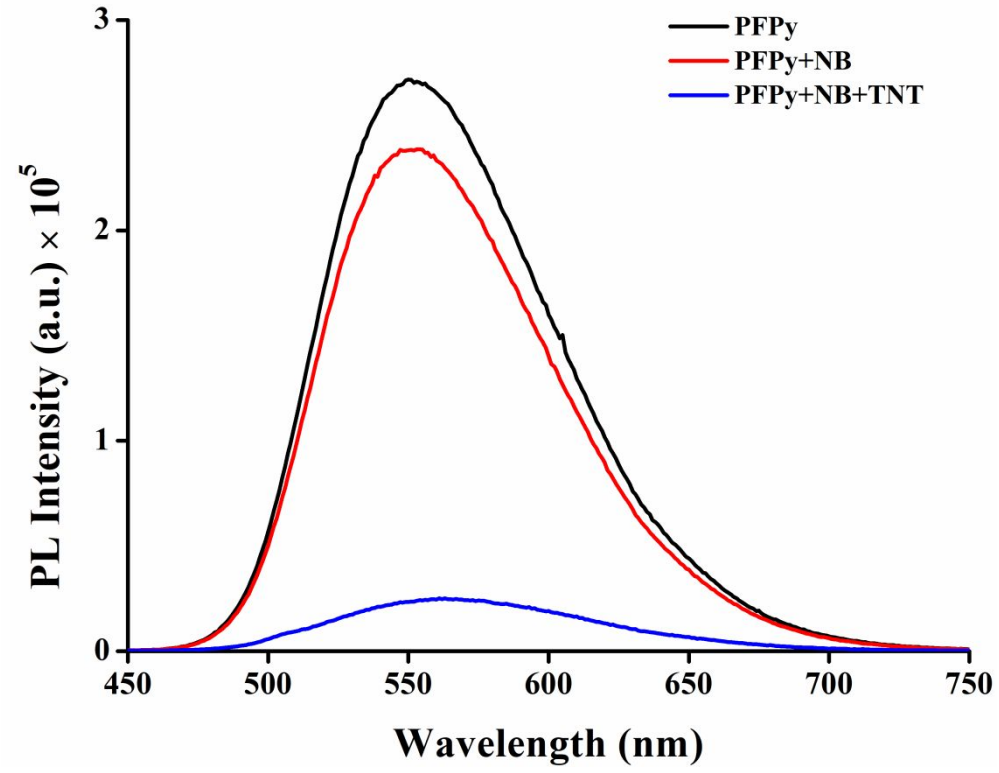


Figure S18. Emission spectra of PFPy (6.66×10^{-6} M) with NB (3.33×10^{-6} M) followed by addition of TNT (3.33×10^{-6} M) in water containing 10 mM-NaOH.

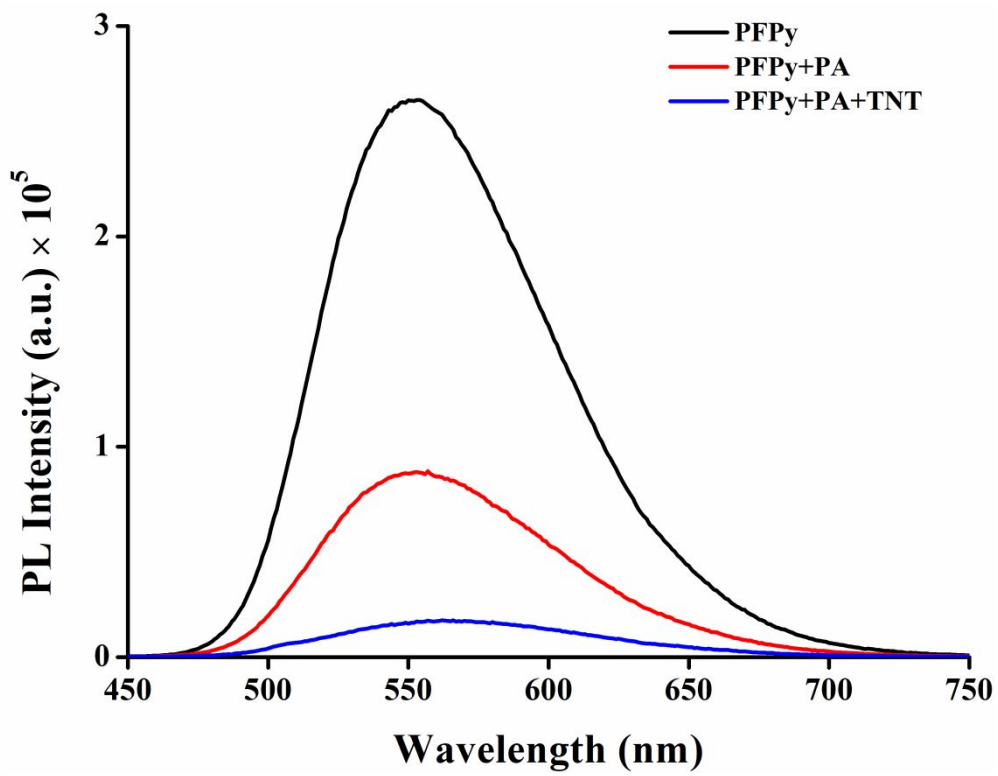


Figure S19. Emission spectra of PFPy (6.66×10^{-6} M) with PA (3.33×10^{-6} M) followed by addition of TNT (3.33×10^{-6} M) in water containing 10 mM-NaOH.

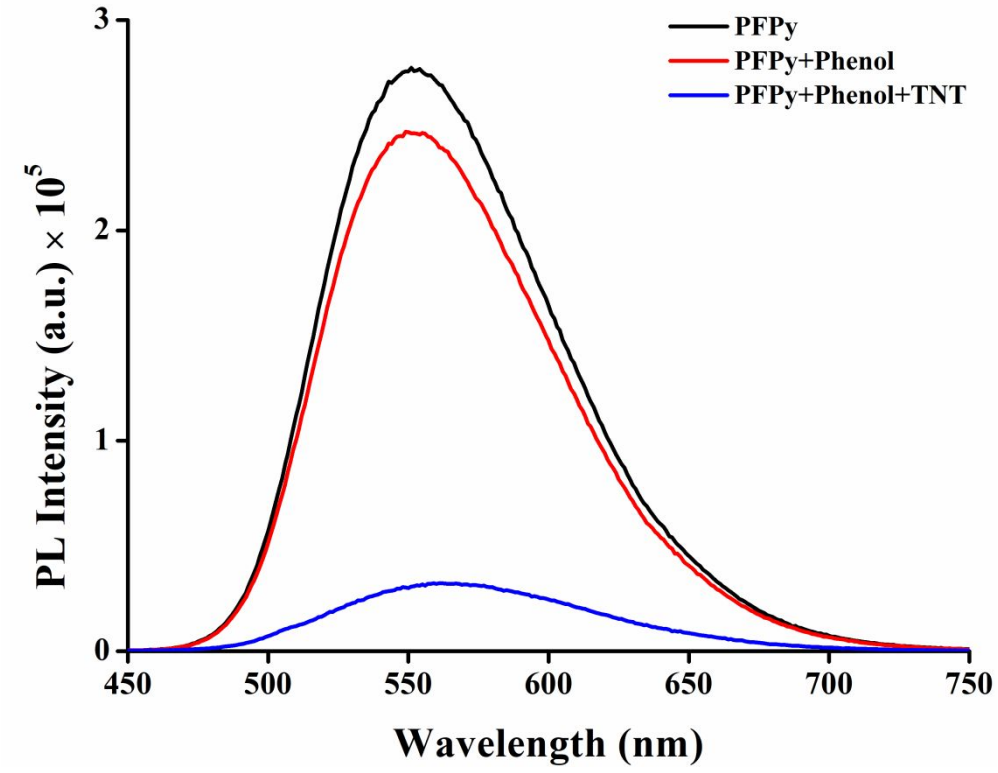


Figure S20. Emission spectra of PFPy (6.66×10^{-6} M) with Phenol (3.33×10^{-6} M) followed by addition of TNT (3.33×10^{-6} M) in water containing 10 mM-NaOH.

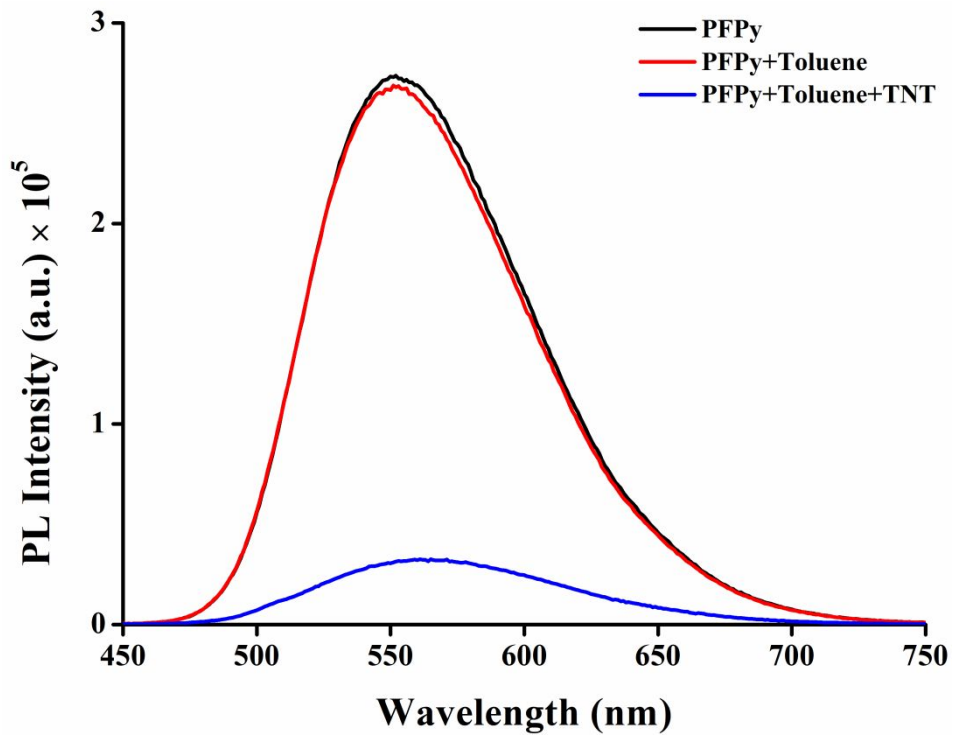


Figure S21. Emission spectra of PFPy (6.66×10^{-6} M) with Toluene (3.33×10^{-6} M) followed by addition of TNT (3.33×10^{-6} M) in water containing 10 mM-NaOH.

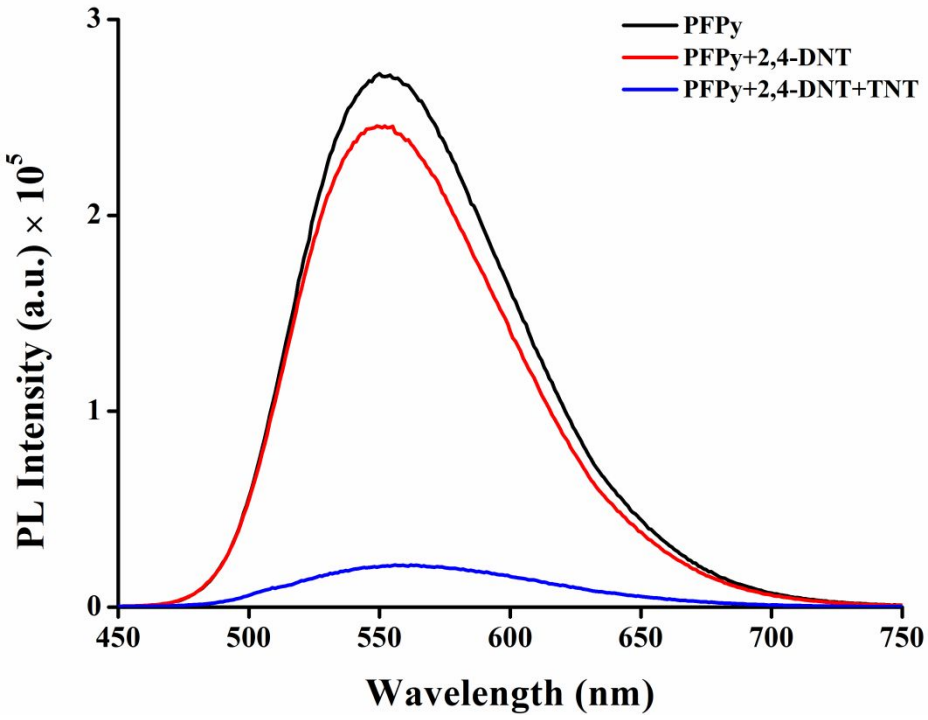


Figure S22. Emission spectra of PFPy (6.66×10^{-6} M) with 2,4-DNT (3.33×10^{-6} M) followed by addition of TNT (3.33×10^{-6} M) in water containing 10 mM-NaOH.

Table S3. Förster distance, Overlap integral J (λ) values and RET efficiency calculated for TNT in water containing 10 mM NaOH.

Solvent	Förster distance R_0 (Å)	J (λ) values ($M^{-1}cm^{-1}nm^4$)	RET efficiency
Water containing 10 mM NaOH	24.95	3.00×10^{13}	35.68

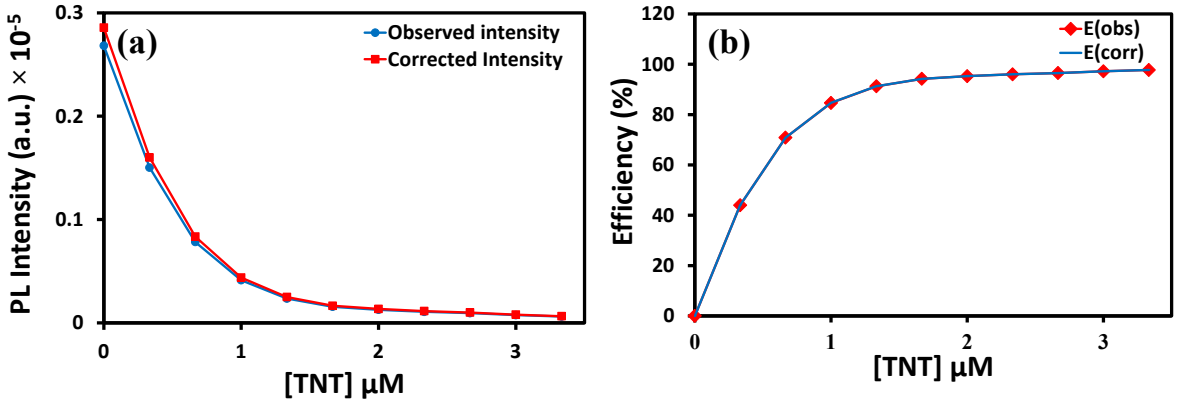


Figure S23. IFE corrections: (a) PL intensity of PFPy (6.66×10^{-6} M) (observed in blue line and after IFE correction in red line) at various concentration of TNT in water containing 10 mM-NaOH. (b) Quenching efficiency (E%= 1- I/I_0) of the corrected (blue line) and observed (red line) measurements for PFPy after addition of various concentration of TNT in water containing 10 mM-NaOH. Where I and I_0 are the fluorescence intensities of PFPy in presence and absence of TNT.

Table S4. Calculations for IFE corrections for quenching of PFPy by TNT in water containing 10 mM-NaOH.

TNT(µM)	A_{ex}	A_{em}	I_{obs}	I_{corr}	I_{corr}/I_{obs} Correction factor (CF)	$I_{corr}/I_{corr,0}$	E_{obs}	E_{corr}
0	0.054454	0.00057	268220.4	285761.5968	1.065398	1	0	0
0.333	0.052787	0.001917	150228.6	159994.3004	1.065006	0.559887	43.99064	44.01127
0.666	0.052831	0.003353	78204.4	83430.18073	1.066822	0.291957	70.84324	70.80427
1	0.049569	0.003158	41220.86	43800.64929	1.062585	0.153277	84.63172	84.67231
1.333	0.048863	0.003661	23498.8	24963.62791	1.062336	0.087358	91.239	91.26418
1.666	0.048467	0.004336	15549.96	16524.59079	1.062678	0.057826	94.20255	94.21735
2	0.047109	0.005027	12661.05	13444.28324	1.061862	0.047047	95.27961	95.29528
2.333	0.046558	0.005566	10686.27	11347.18519	1.061847	0.039709	96.01586	96.02914
2.666	0.045965	0.006014	9394.993	9974.380971	1.06167	0.034905	96.49729	96.50954
3	0.045776	0.006612	7488.206	7953.746795	1.06217	0.027834	97.20819	97.21665
3.333	0.045173	0.006894	6063.571	6438.162491	1.061777	0.02253	97.73933	97.74702

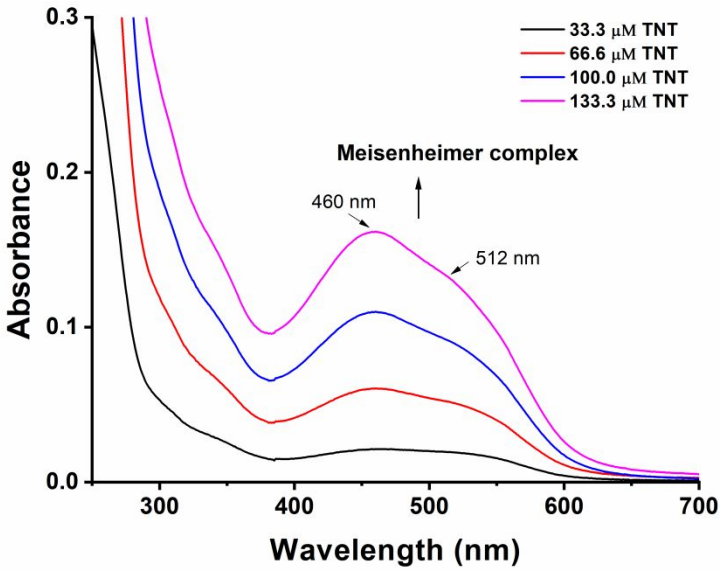


Figure S24. Absorbance of TNT (excess) in the water containing 10 mM NaOH to realize a clear Meisenheimer complex peak formation of TNT.[1-5]

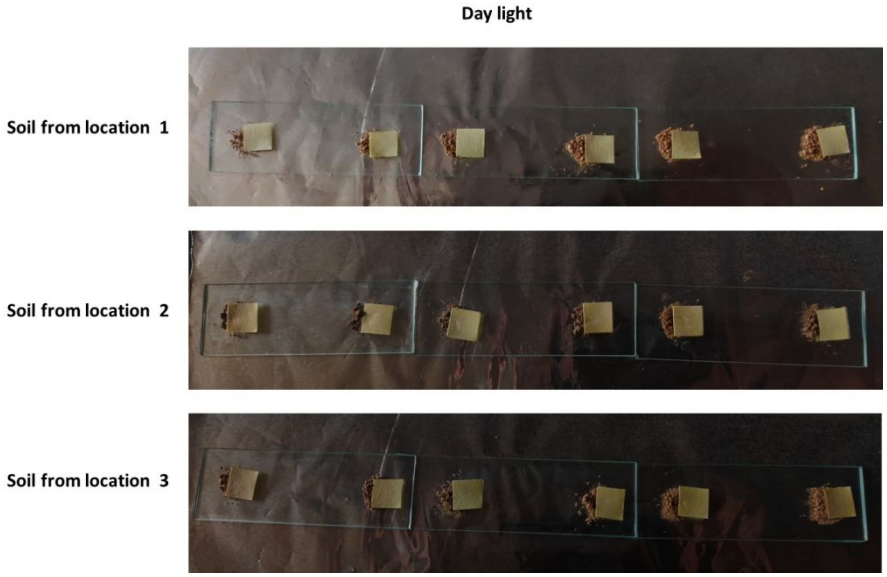


Figure S25. Photographs of portable paper strips under day light while contacting with soil samples already spiked with different concentration of TNT.

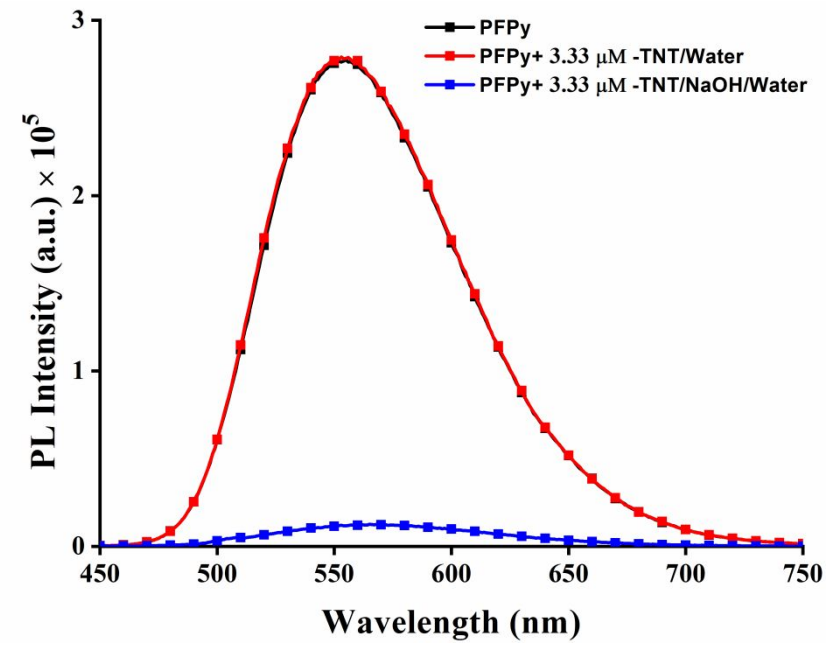


Figure S26. Emission spectra of PFPy (6.66×10^{-6} M) in water (black), PFPy (6.66×10^{-6} M) with TNT (3.33×10^{-6} M) in water (red) and PFPy (6.66×10^{-6} M) with TNT (3.33×10^{-6} M) in water containing 10 mM-NaOH (blue).

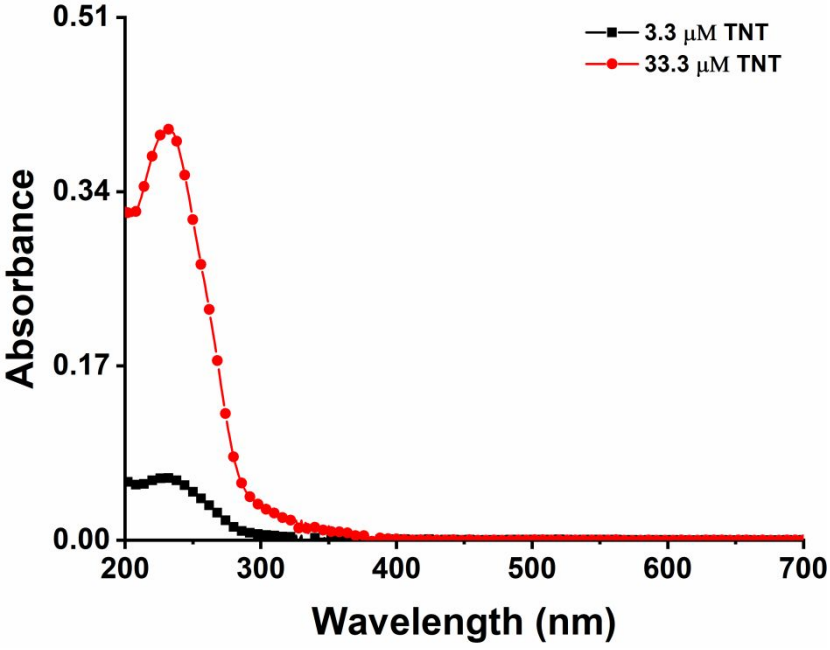


Figure S27. Absorbance spectra of TNT at different concentrations (3.33×10^{-6} M and 33.3×10^{-6} M) in water at neutral pH.

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