Supporting Information

Precise aggregation-induced emission enhancement *via* H^+ sensing and its use in ratiometric detection of intracellular pH values

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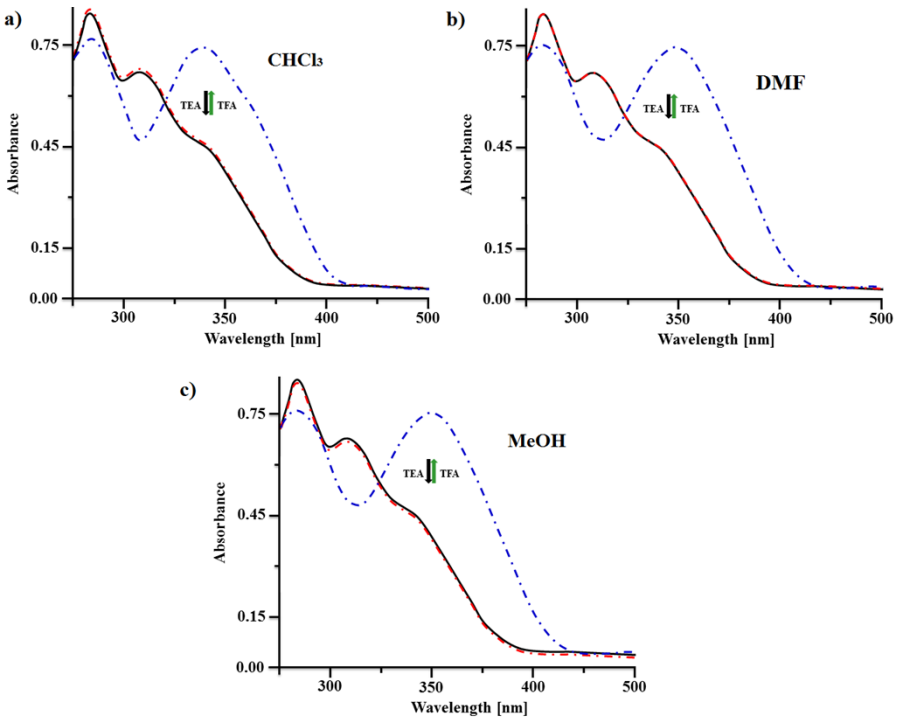


Fig. S1 UV-vis absorption spectral changes of Py-TPE **1** (1×10^{-5} M): with and without addition of 0-10 equiv. TFA (10^{-4} M) in in $CHCl_3$, DMF, and MeOH, respectively, and upon addition of TEA absorption restored to its original position identical to Py-TPE **1**.

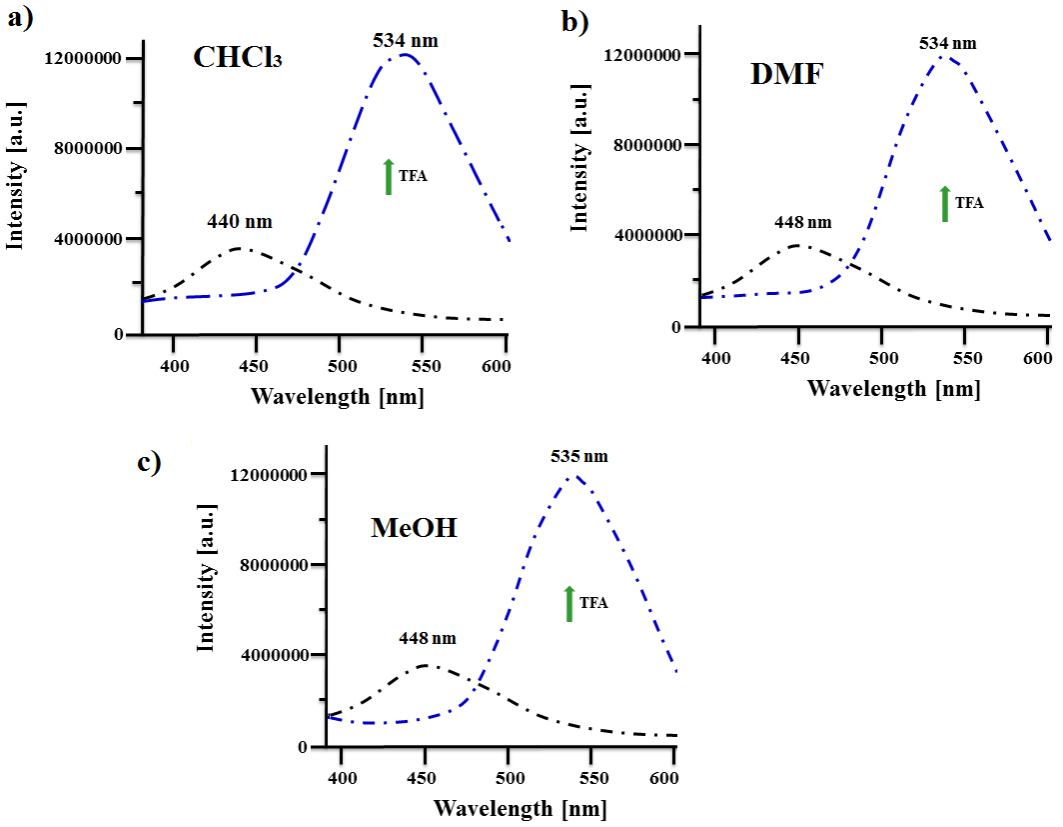


Fig. S2 Fluorescence emission spectra of **1** (1×10^{-5} M): with and without addition of 10 equiv. of TFA (10^{-4} M) in $CHCl_3$, DMF and MeOH, respectively.

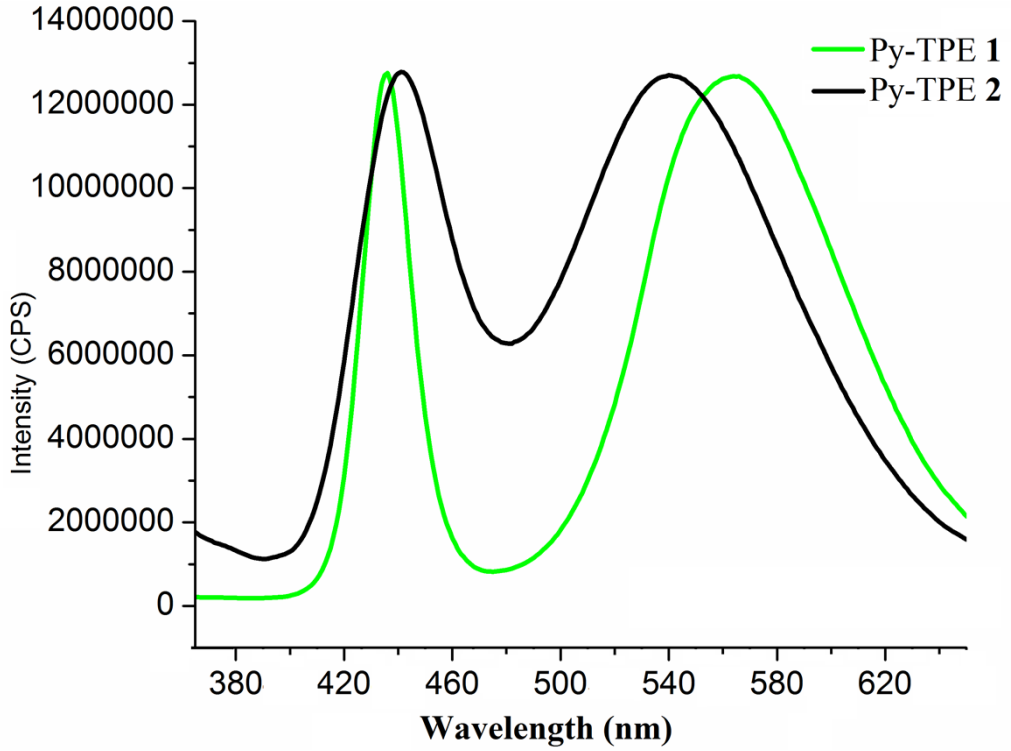


Fig. S3 Fluorescence emission spectra (λ_{ex} = 365 nm) of **1** and **2** in solid state with and without protonation, respectively.

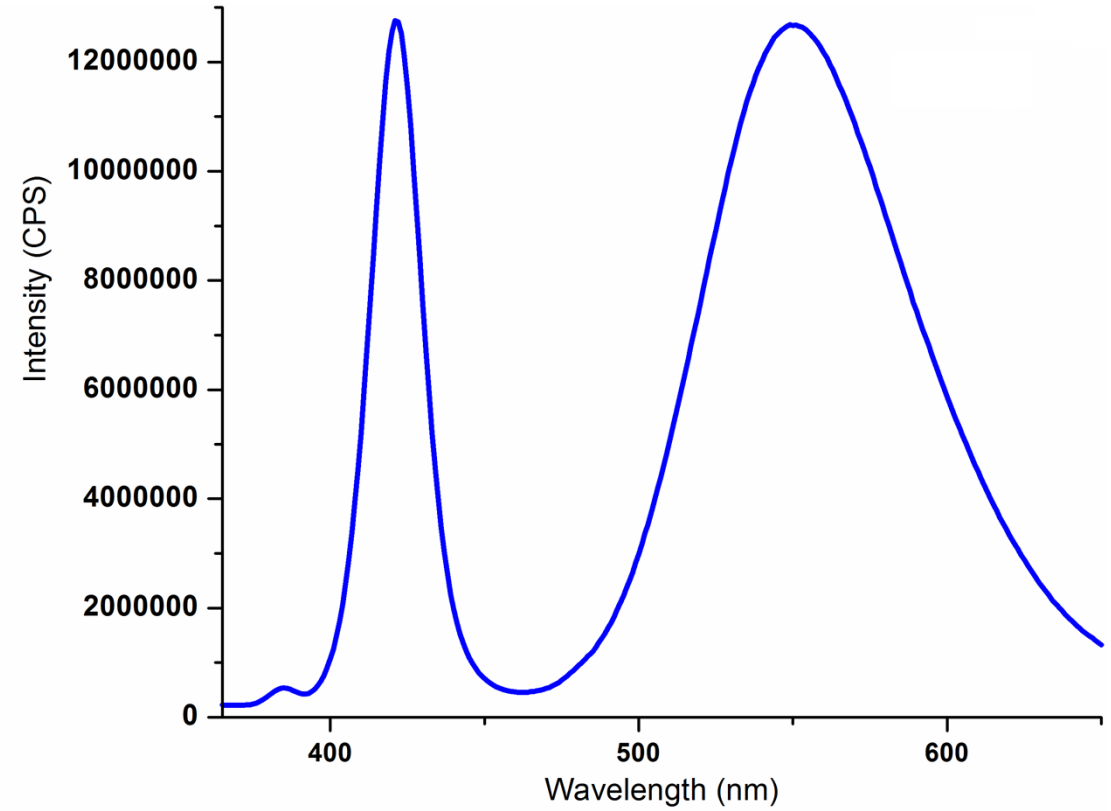


Fig. S4 Fluorescence emission spectra (λ_{ex} = 365 nm) of **1** in water without protonation.

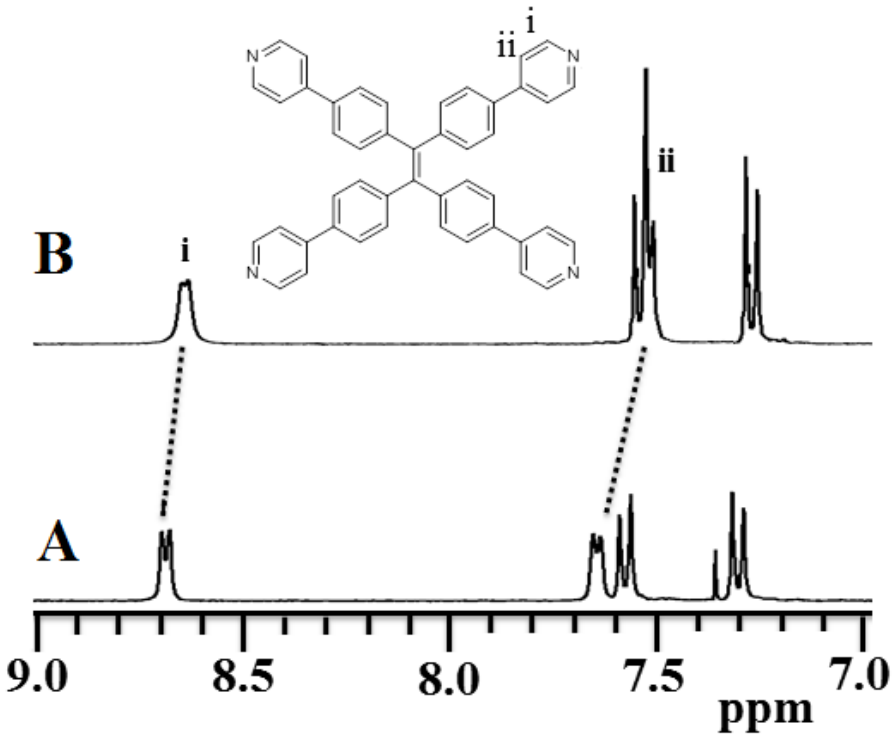


Fig. S5 Partial ^{1}H NMR spectra of Py-TPE **1** with TFA (A) and without TFA (B).

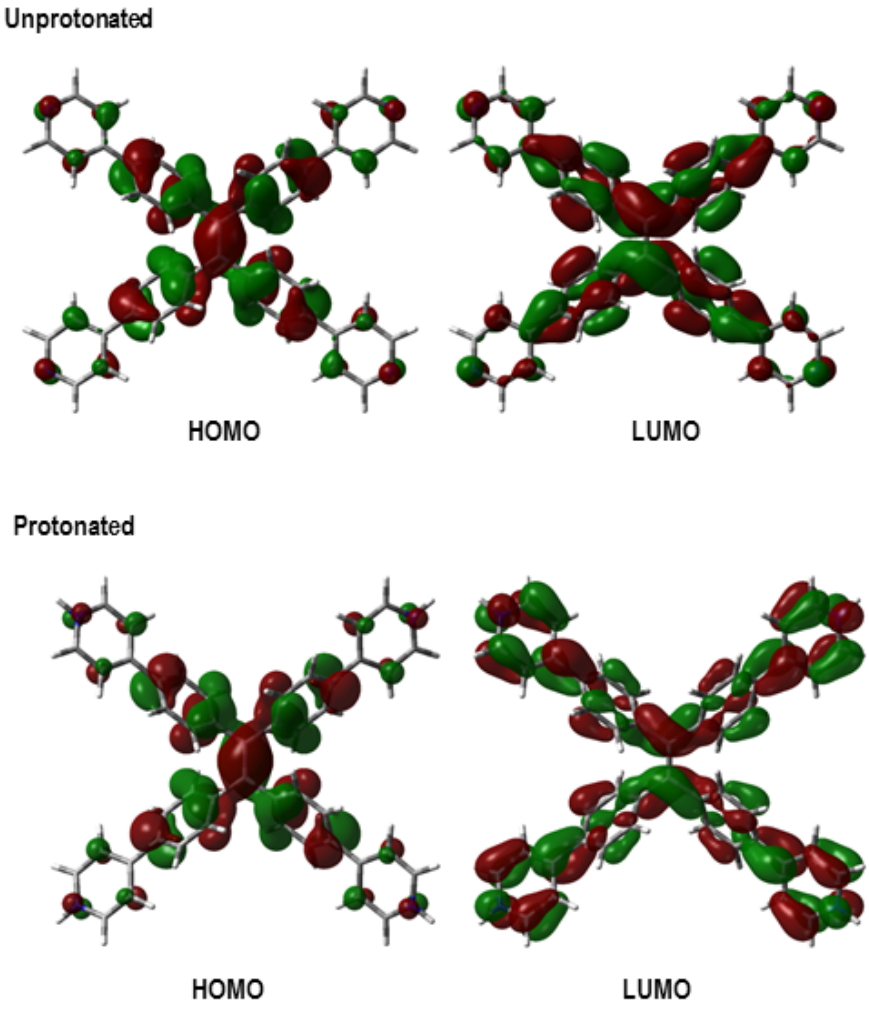


Fig. S6 Orbital density distribution for the HOMOs and LUMOs of Py-TPE **1** (upper) and Py-TPE **2** (lower). Density functional theory calculations were performed on **1** and **2** using the Gaussian 09 suite of programs and B3LYP/6-31G level of theory.

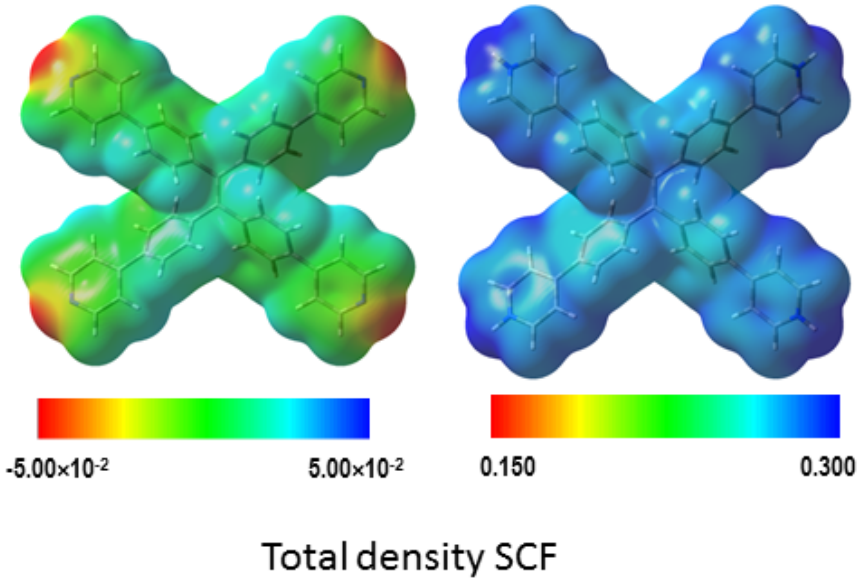


Fig. S7 Total density SCF

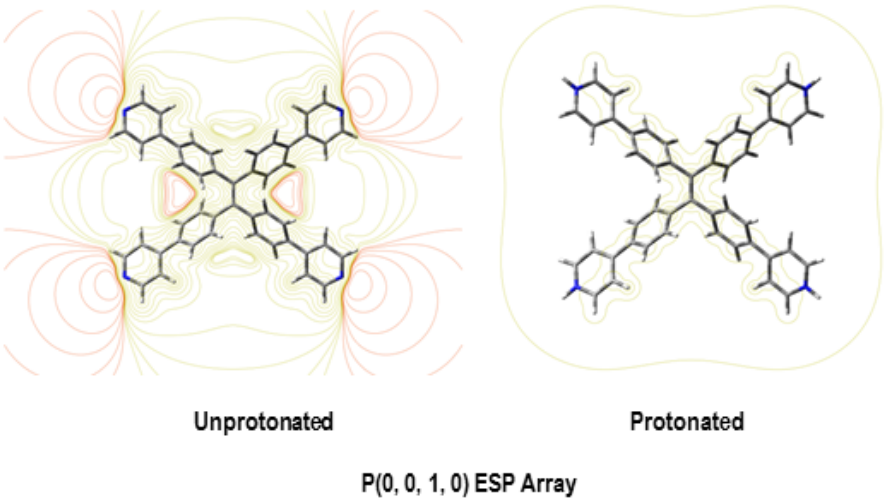


Fig. S8 ESP Array P (0, 0, 1, 0)

Table S1. Comparative DFT calculation of **1** and **2**

	Unprotonated	**Protonated**
Calculation Type	FOPT	FOPT
Calculation Method	RB3LYP	RB3LYP
Basis Set	6-31G	6-31G
Charge	0	+4
Spin	Singlet	Singlet
Total Energy (a.u.)	-1990.67974909	-1991.97389922
RMS Gradient Norm (a.u.)	0.00000371	0.00000096
Dipole Moment (Debye)	0.0001	0.0013
HOMO (Hartree)	-0.21228	-0.50574
LUMO (Hartree)	-0.07954	-0.39475
HOMO-LUMO gap (Hartree)	0.13274	0.11099
HOMO-LUMO gap (eV)	3.612041	3.020193
HOMO-LUMO gap (nm)	343.2525	410.5175

Table S2. TD-DFT calculation of **1** excited states

g→e	Contribution to the CI wave function	Transition energy	Oscillator strength f	S^2
^{3}A		2.2141 eV (559.96 nm)	0.0000	2.000
152→169	0.1029			
168→169	0.65307			
168→175	0.14298			
^{3}A		3.0417 eV (407.62 nm)	0.0000	2.000
161→170	-0.1895			
162→172	0.16955			
163→169	0.24388			
167→169	0.2439			
168→171	0.47461			
168→183	0.1021			
^{3}A		3.0437 eV (407.35 nm)	0.0000	2.000
161→171	-0.20068			
162→169	0.29089			
163→172	0.12825			
167→172	0.1266			
168→170	0.50558			
168→182	-0.10455			
^{1}A		3.1957 eV (387.97 nm)	0.6433	0.000
168→169	0.70544			
^{3}A		3.2007 eV (387.37 nm)	0.0000	2.000
152→172	-0.10399			
161→169	-0.3365			
162→171	0.25883			
163→170	0.20219			
167→170	0.20219			
168→172	0.34963			
168→184	0.10537			
^{1}A		3.6117 eV (343.28 nm)	0.4656	0.000
162→169	-0.10298			
168→170	0.69526			
^{1}A		3.7570 eV (330.01 nm)	0.0087	0.000
168→171	0.70102			
^{1}A		3.7765 eV (328.30 nm)	0.0203	0.000
163→169	0.25784			
167→169	0.62166			
168→173	-0.11489			

Table S3. TD-DFT calculation of **2** excited states

g→e	Contribution to the CI wave function	Transition energy	Oscillator strength f	S^2
^{3}A		2.0480 eV (605.39 nm)	0.0000	2.000
168→169	0.63398			
168→177	-0.22748			
^{3}A		2.4831 eV (499.32 nm)	0.0000	2.000
161→171	0.11319			
164→172	0.10842			
167→169	-0.17346			
168→170	0.63626			
^{3}A		2.4893 eV (498.07 nm)	0.0000	2.000
161→170	0.10958			
164→169	-0.15956			
167→172	0.11425			
168→171	0.64099			
^{1}A		2.6669 eV (464.91 nm)	0.6337	0.000
168→169	0.70652			
^{3}A		2.6740 eV (463.67 nm)	0.0000	2.000
161→169	-0.15258			
164→170	0.17178			
166→169	-0.1488			
167→171	0.18574			
168→172	0.59572			
^{1}A		2.8111 eV (441.05 nm)	0.0238	0.000
168→170	0.70571			
^{1}A		2.8275 eV (438.50 nm)	0.5382	0.000
168→171	0.70486			
^{1}A		3.0830 eV (402.16 nm)	0.0000	0.000
168→172	0.6964			

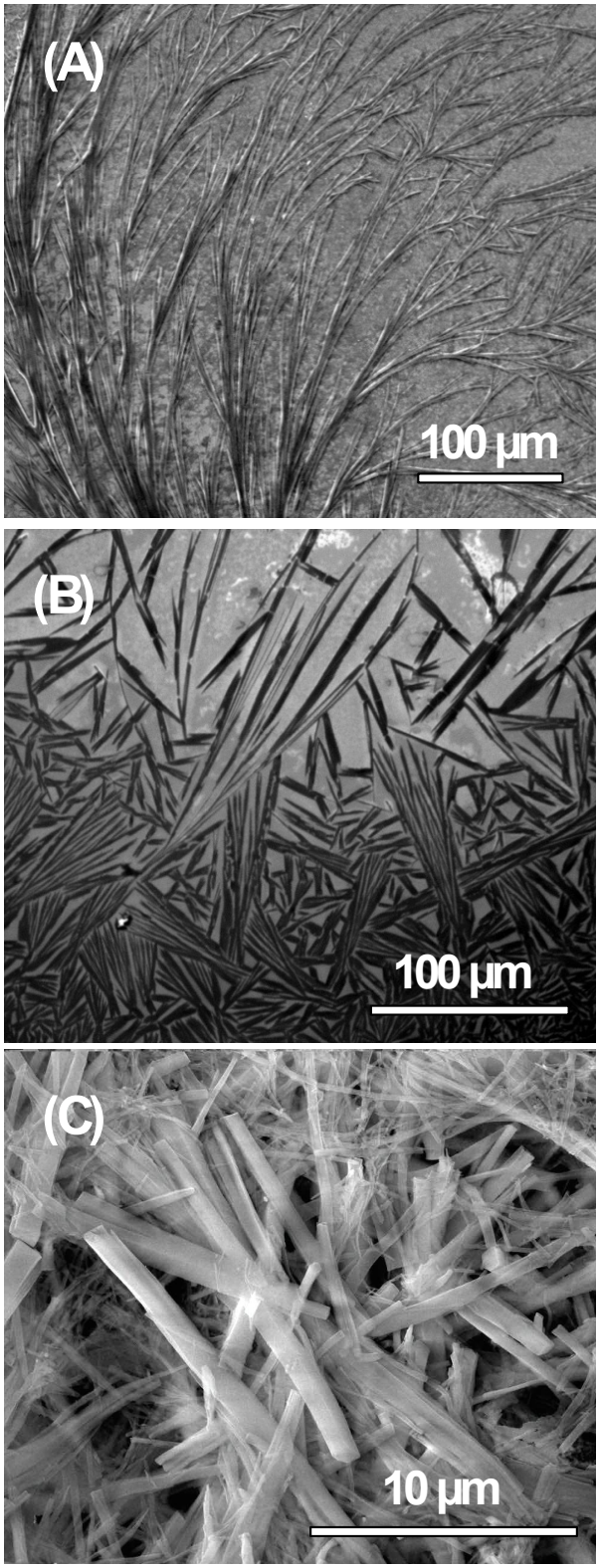


Fig. S9 Scanning electron micrographs of recrystallized **2** (10^{-4} M) fractal formed by solvent evaporation from (A) DMF, (B) MeOH and (C) $CHCl_3$ solutions.

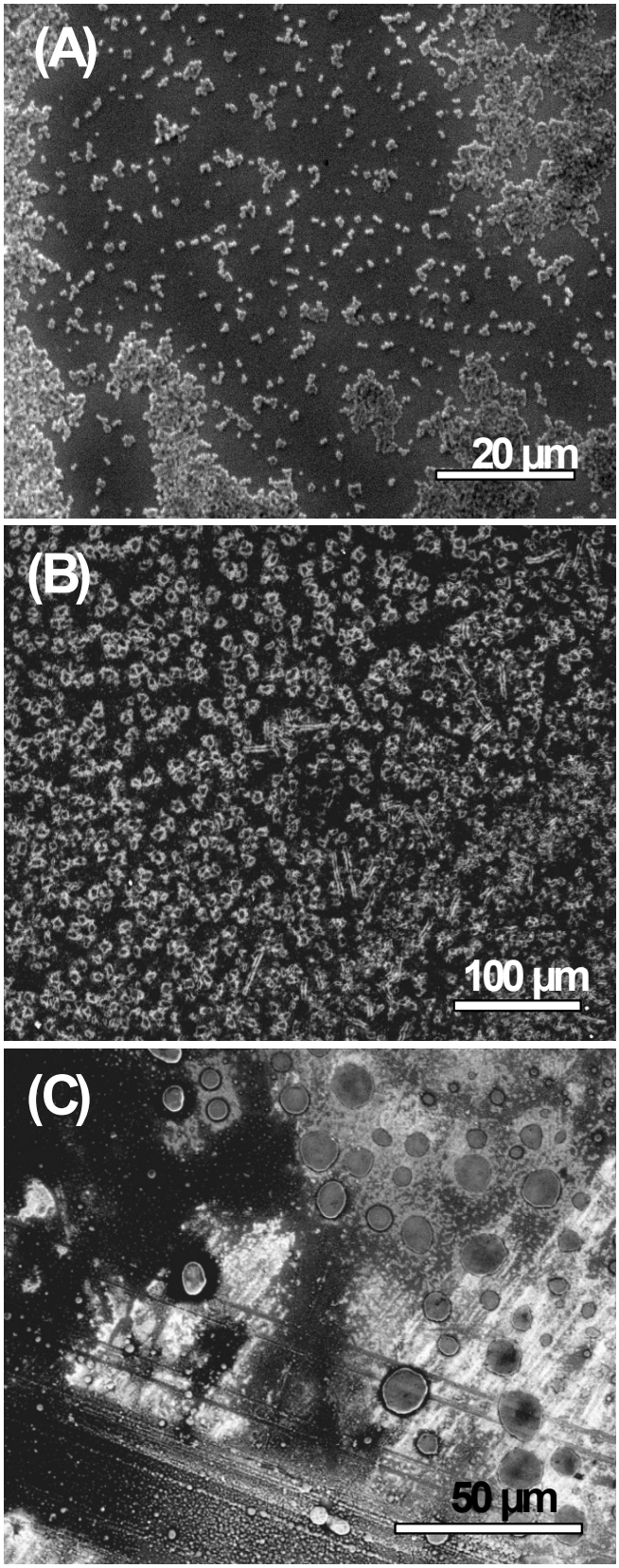


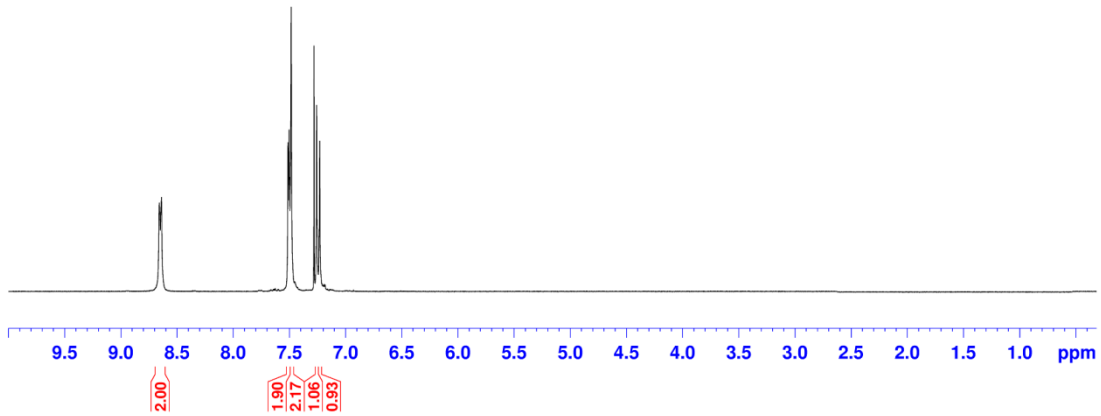
Fig. S10 Scanning electron micrographs of **1** (10^{-5} M) after solvent evaporation from: (A) MeOH, (B) DMF, and (C) $CHCl_3$ solutions, forming non-crystalline global supramolecular aggregates.

Materials & Methods for living cells

RPMI media, foetal bovine serum, phosphate buffered saline (PBS) and penicillin-streptomycin were obtained from Life Technologies (USA) and dimethyl sulfoxide (DMSO) was obtained from Sigma-Aldrich (USA). A stock solution of 500µg/mL of the dye was prepared in DMSO and used for all cell culture experiments.

Prostate cancer (PC-3) cells were maintained in RPMI media with 10% foetal bovine serum, 100U/mL penicillin and 100U/mL streptomycin, at 37°C and 5% CO_2. 2 x 10^5 cells were seeded into 24 well plates for 24 hours, after which cells were treated with 5µg/mL dye for 2 hours. Cells were washed with cold to remove unreacted dye. The fluorescence staining of the dye was exposing cells to in acidic (pH3), neutral (pH 7) and alkaline (pH9) environments, and fluorescence of the cells was observed using a Nikon Eclipse TS100 microscope fitted with mercury lamp.

^{1}H NMR of Py-TPE **1**



^{13}C NMR of Py-TPE 1