Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B

Supporting Information

Aggregation Induced Chirality in a Self Assembled Perylene Based Hydrogel: Application of the Intracellular pH Measurement

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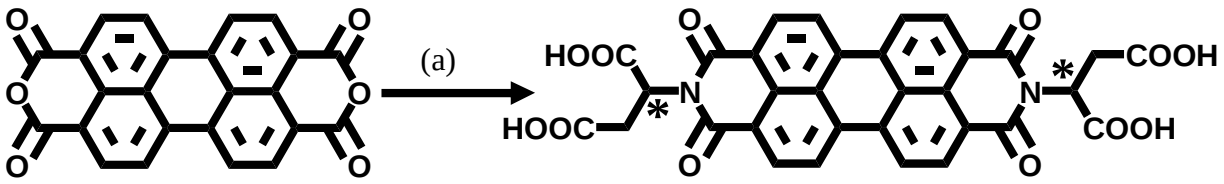
1) Materials and methods

Reagents were purchased from sigma-aldrich and used as received. DAPI and LAPI had been synthesized using literature procedure.[1] For all aqueous mixtures and for spectroscopic studies, water of spectroscopic or HPLC grade was used.

^{1}H-NMR spectra were recorded at room temperature on 300 and 500 MHz spectrometers (Bruker). ^{1}H NMR chemical shifts (δ) were reported in parts per million (ppm) ^{1}H NMR shifts were referenced to the residual hydrogen peak of D_2O (4.60 ppm). Splitting patterns were denoted *s* (singlet), *br*. (broad). Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry was carried out with Bruker Daltonics FLEX-PC using diathranol as a matrix. FT-IR spectra were recorded using KBr pellets of samples in an FTIR-8400S instrument (Shimadzu).

2) Synthesis and characterization of API



when a=L-aspartic acid,API=LAPI
when a=D-aspartic acid,API=DAPI

Scheme S1. Synthesis of perylene derivatives. Reagents and conditions: (a) DMSO, H_2O, 100^0C/aspartic acid, KOH, H_2O, 3 h, 68%.

Synthesis of *N,N'*-Bis[aspartic acid]-3,4,9,10-perylenetetracarboxylic diimide (API):

0.5 g (1.3 mmol) of perylene-3,4:9,10-tetracarboxylic dianhydride was suspended in 50 mL of DMSO and heated to 100 °C in a reflux apparatus. Aspartic acid (4.2 g; 32 mmol) was dissolved in 8 mL of 8M KOH solution and added dropwise to the suspension. The mixture was stirred at 100 °C for 3 hours, and then cooled to room temperature. The residue was collected by filtration, and washed with a mixture of DMSO/water (2:1). To remove the DMSO, the residue was dissolved in a minimum amount of hot water and precipitated by the addition of acetone.

The solid material was then collected by filtration, washed twice with acetone, and dried under vacuum to the desired product. (Yield: 68%).

^{1}H-NMR of API (300 MHz , D_2O, 25^{0}C) ∂ : 8.17 (br,4H), 7.92 (br,4H), 5.84 (br , 2H), 3.18 (d, 2H), 2.94 (d, 2H). ^{13}C-NMR of API ∂ : 179.95, 176.58, 164.25, 131.30, 122.97, 121.75, 54.25, 38.69.

FT-IR (KBr) ν_{max} = 748, 804, 859, 1124, 1252, 1355, 1592, 1642, 1690, 3433 cm^{-1}.

MS (MALDI-TOF): m/z calc. for $C_{32}H_{18}N_2O_{12}$: 622.09, found: 699.56 [$M+2K^+$].

UV/Vis (H_2O): λ_{max}/nm ($\varepsilon/M^{-1}cm^{-1}$) 532 (27634), 496 (19673) , 465 (9268).

Fluorescence (H_2O): λ_{max}/nm: 548, 589, Fluorescence quantum yield (Φ_f) = 0.64.

3) Instruments and Experimental Techniques

3.1. Optical Measurements

The UV-Vis spectra of all samples were studied with Hewlett-Packard UV-Vis spectrophotometer (model 8453). The CD spectra of all the solutions were taken in a spectro polarimeter (JASCO, model J-815) in a 1 mm quartz quvette. Temperature dependent CD studies were carried out in a 1mm quartz cuvette with a thermistor directly attached to the wall of the cuvette holder at a heating rate of 2 °C /min. Fluorescence studies of solution and hydrogel samples, prepared in a sealed cuvette, were carried out with Horiba Jobin Yvon Fluoromax 3 instrument at excited wavelength 490 nm. The quantum yields of fluorophore were determined by using the Parker-Rees method (S2, S3) and using *Rhodamine 6G* solution in water (λ_{abs}= 536 nm, λ_{em}= 550 nm, φ = 0.95) as reference.

3.2. Field Emission-Scanning Electron Microscope (FE-SEM)

To understand the morphology of the gel, small portions of the hydrogels were placed on glass cover slip and were dried in air at room temperature, finally in oven at 60^0C. It was coated with platinum for 60 sec prior to observation through a FESEM instrument (JEOL, JSM 6700F) operating at 5 KV.

3.3. Atomic Force Microscopy (AFM)

AFM studies were conducted using atomic force microscopy (Veeco, model AP0100) in noncontact mode at a tip resonance frequency of 300 kHz. Samples for the imaging were prepared by drop

casting the **API gel** (pH 4.00) solution on freshly cleaved mica surface at the required concentrations at ambient conditions.

3.4. NMR of gel :

^{1}H NMR of solution and gel were performed at room temperature on 500 MHz spectrometers (Bruker) using a solution of API in D_2O at a concentration of 5 x 10^{-3} M. Subsequently 50 µl of 0.2 (N) DCl in D_2O (35 %) measuring the pH of the solution as 3.05.

3.5. Fluorescence lifetime measurement:

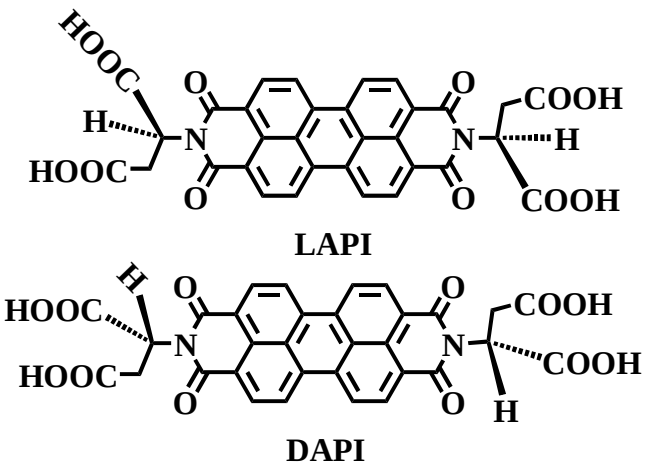
Fluorescence lifetimes were measured by using a time-correlated single photon counting fluorometer (Fluorecule, Horiba Jobin Yvon). The system was excited with a 490 nm NanoLED from Horiba Jobin Yvon having λ_{max} at 550 nm and 665 nm with a pulse duration of <200 ps. All solutions prepared at room temperature (25°C) were deoxygenated by purging with argon gas for 30 min prior to measurement.

3.6. pH measurement:

It was measured using probe type pH-meter (IQ Scientific Instruments, Model-IQ240, Benchtop/Portable pH Meter with ISFET probes) which were calibrated with buffer of pH = 7.00 and pH = 4.00 prior to each measurement.

4) Molecular structure and additional photos

(a)



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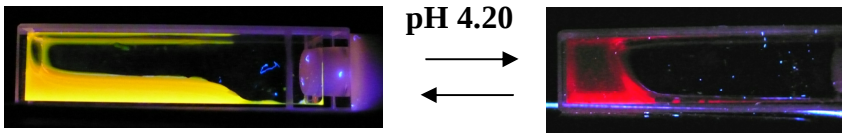


(c)

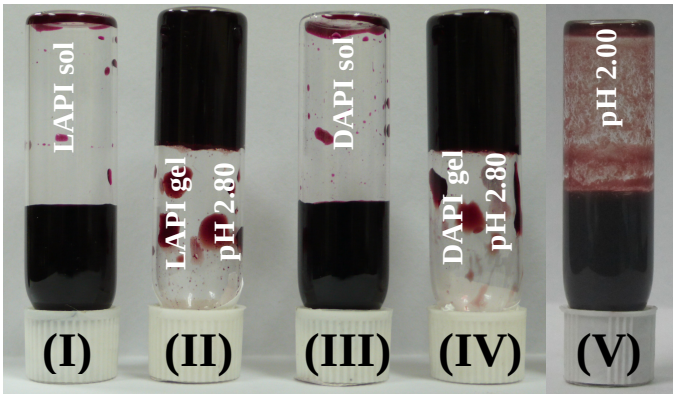
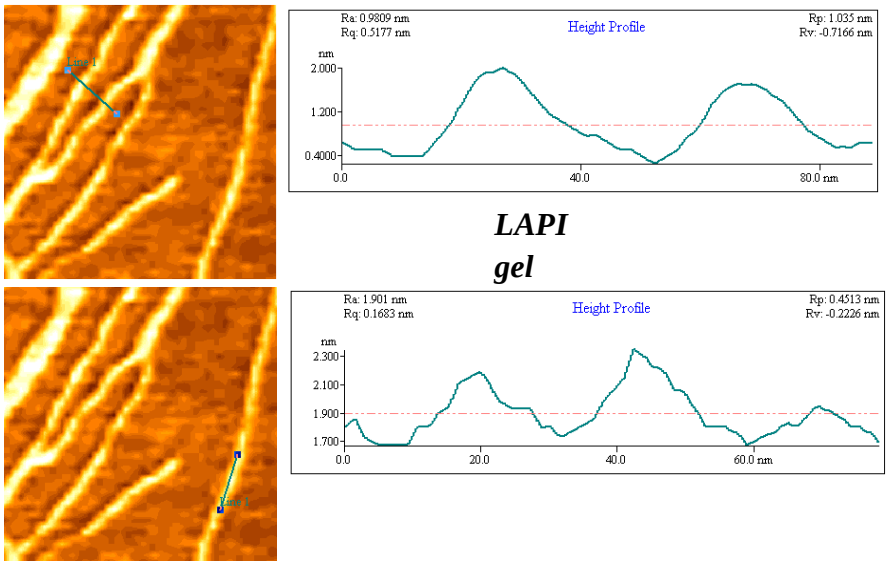


Fig. S1 (a) Molecular structure of LAPI, and DAPI, (b) Reversible fluorescence image of LAPI solution and gel ([LAPI] = 2.5 x 10^{-4}M) under UV light of 365 nm, and (c) Photo of LAPI solution (I)([LAPI]=10^{-2}M), LAPI hydrogel (II), DAPI solution ([DAPI]=10^{-2}M) in water (III), Photo DAPI hydrogel (IV) and API forms precipitate at pH 2.00 (V).

5) AFM height images

(a)



(b)

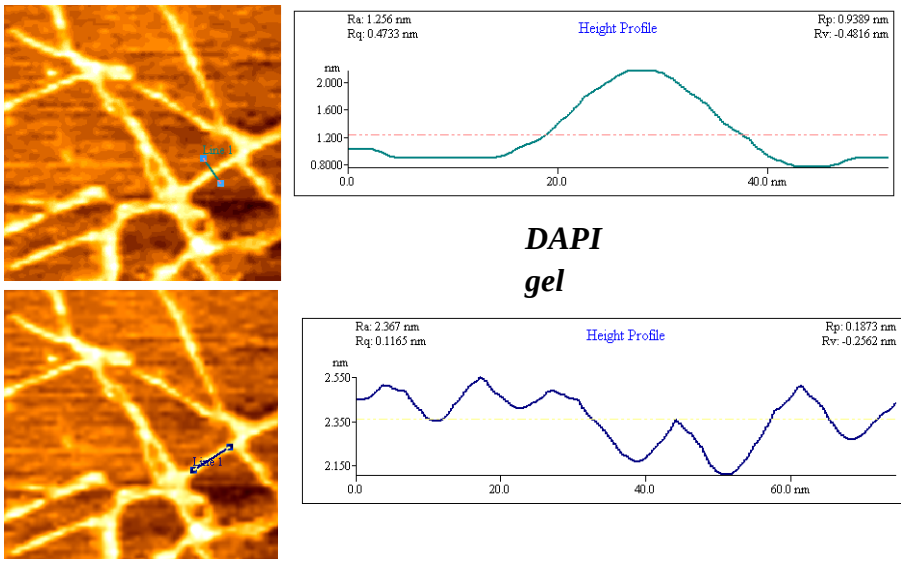


Fig. S2 AFM topolography observed on mica surface, showing twisted helical fiber and height profile, (a) for LAPI gel ([LAPI]= 2.5×10^{-4} M) and for (b) DAPI gel ([DAPI]= 2.5×10^{-4} M).

6) Concentration dependent UV-Vis absorption results

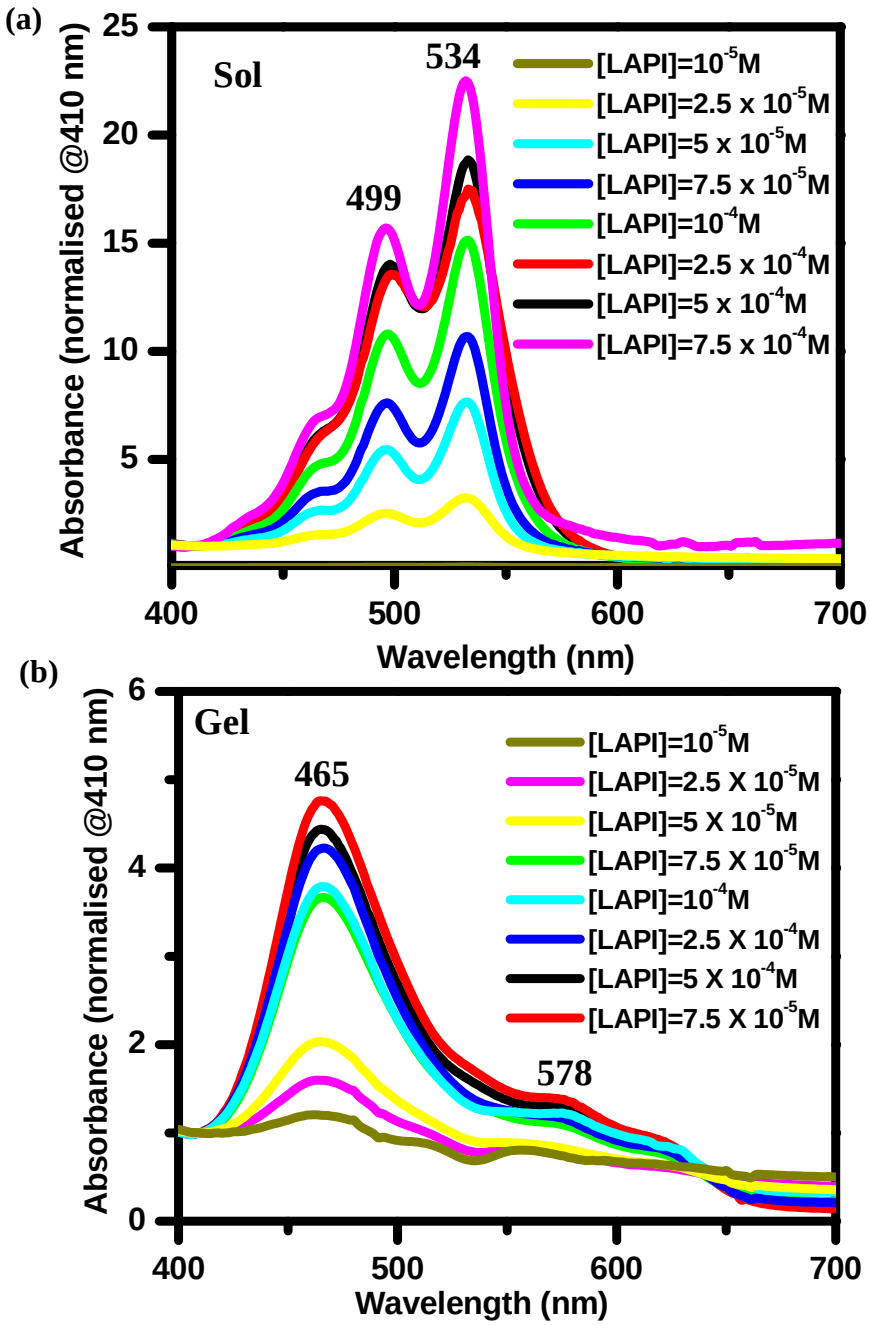


Fig. S3 Concentration dependent UV-Vis spectra of (a) LAPI sol and (b) LAPI gel which was prepared at pH 4.00. The spectra were collected at 20^0C using path length 1.00mm. Spectra are revealing the different aggregation of LAPI in the solution and the gel.

7) pH dependent UV-Vis spectra of LAPI systems

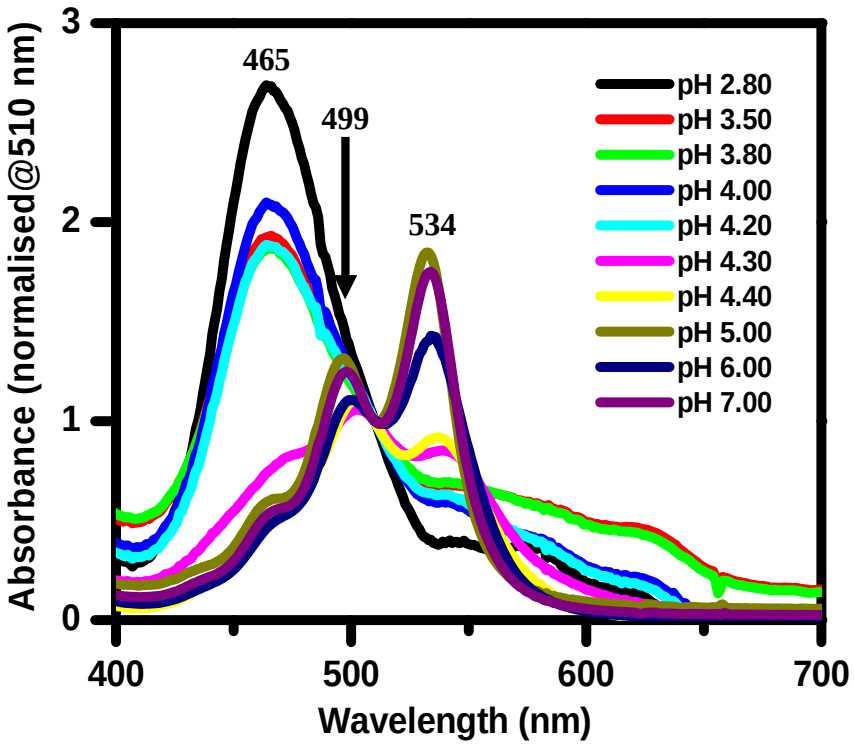


Fig. S4 pH dependent UV-Vis spectra of LAPI system (10^{-4} M) at room temperature.

8) pH dependent photoluminescence studies of LAPI systems

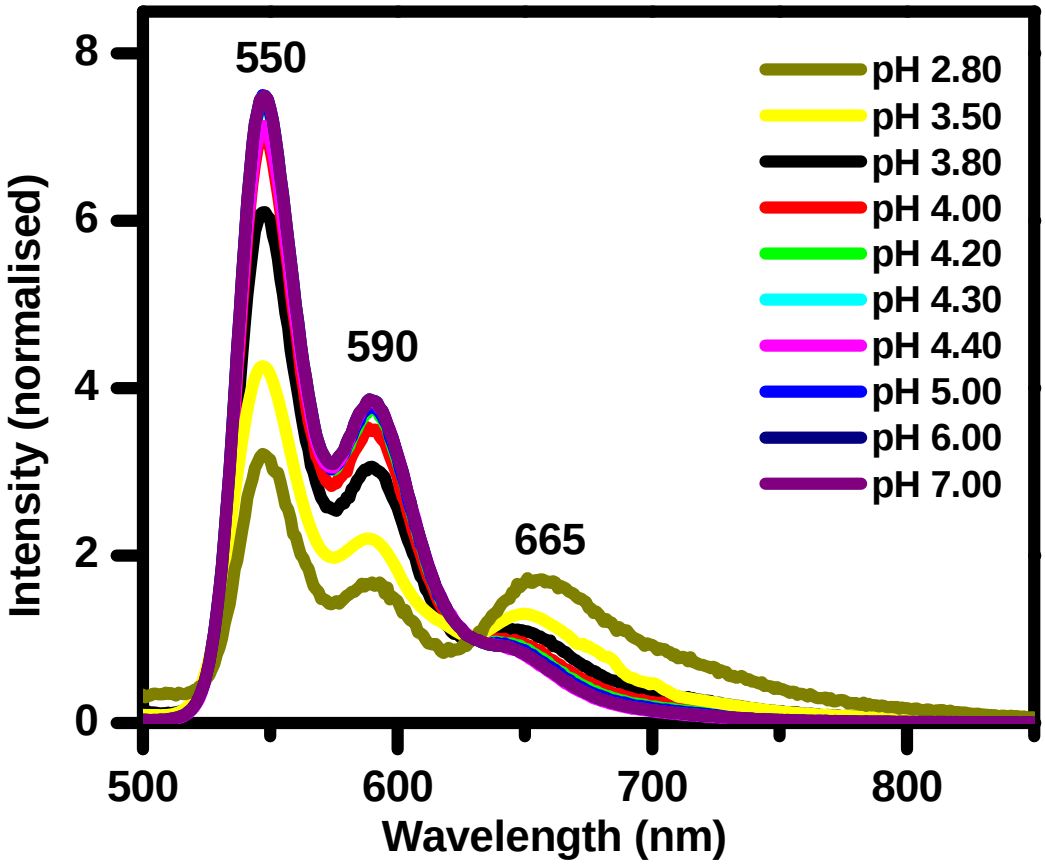


Fig. S5 pH dependent fluorescence spectra of LAPI (10^{-4} M) at room temperature using λ_{ex}=490 nm, slit-2/2 nm and pathlength 10.00 mm. The generation of new emission peak at 665 nm indicates J-type aggregation of perylene core. Two types of aggregation of chromophores are reported in the literature. First one is H type aggregation where face to face stacking occurs between the chromophores and can be monitored by the blue shifting of λ_{max} either in absorbance or in fluorescence with respect to monomer. In J type of aggregation red shifting occurs in the absorbance or fluorescence spectra and there is face to face slippage between the chromphore units. In our system after formation of gel, both UV-Vis spectra and the fluorescence spectra reveal the red shifting of absorption or emission peak revealing the formation of J-type aggregates [ref. 11 of main text].

9) Fluorescence lifetime results:

Table S1: Fluorescence decay times (τ, ns) and their relative amplitude (α) obtained from fluorescence decay profile. The average life time has calculated using the equation.[3,4]

$$\langle \tau \rangle_{adv} = \frac{\alpha_1\tau_1 + \alpha_2\tau_2 + \alpha_3\tau_3}{100}$$

	λ_{em} (nm)	τ_1 (ns)	τ_2 (ns)	τ_3 (ns)	α_1(%)	α_2(%)	α_3(%)	<τ> (ns)
LAPI solution	645	0.20	3.94	-	-0.86	100.86	-	3.97
DAPI solution	645	1.09	3.88	0.24	3.19	93.51	3.30	3.67
LAPI gel	675	0.59	1.26	0.37	32.09	58.08	9.03	1.14
DAPI gel	675	0.64	1.34	0.58	34.09	56.06	9.05	1.02

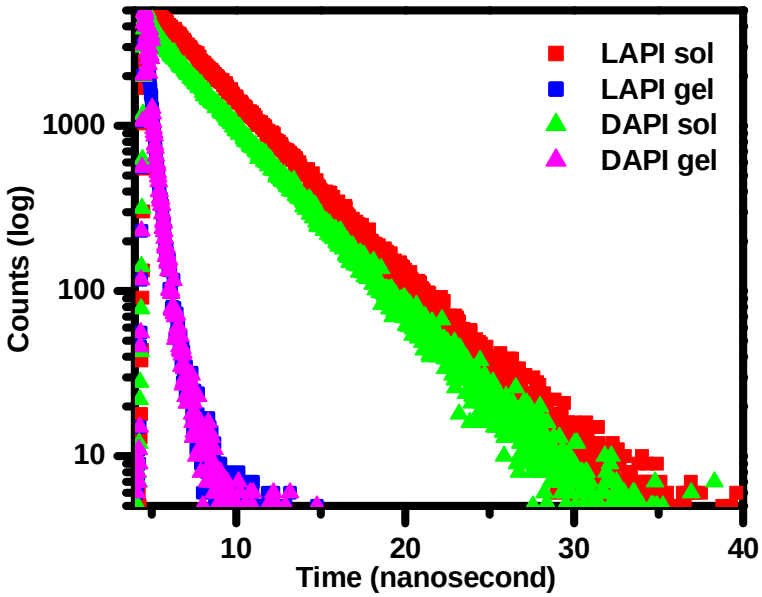


Fig. S6 Time-resolved fluorescence decay of LAPI solution (red), DAPI solution (green), LAPI hydrogel (blue), and DAPI hydrogel (magenta) at 25^0C (λ_{ex} = 440 nm). The sharp profiles on the left are the lamp profile. The emission decay profile observed with nanosecond time-resolved fluorescence shows the decrease of life time in the gel state (1.14 ns) with respect to LAPI solution (3.97 ns), indicative of quenching of fluorescence intensity in gel state.

10) Concentration dependent CD results of DAPI systems

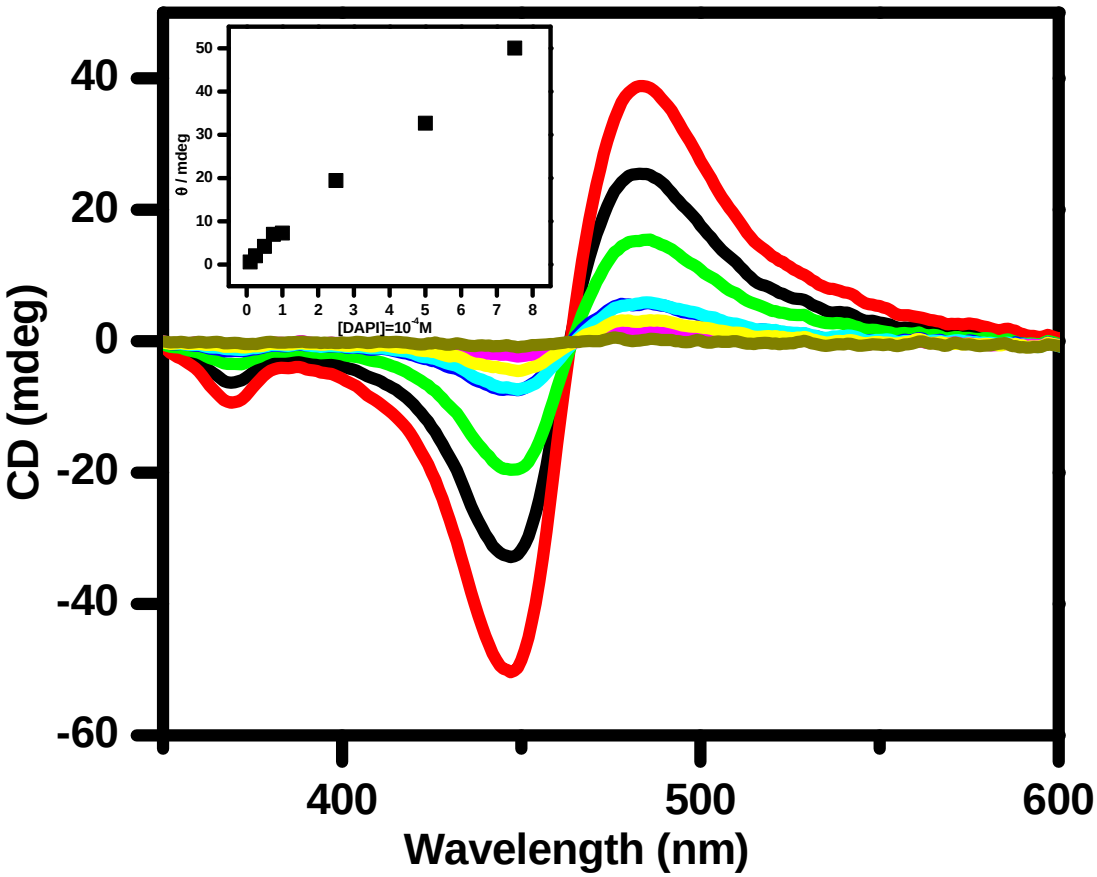


Fig. S7: Concentration dependent CD spectra of **DAPI** (pH = 4.0) at 20^0C at different concentration, [DAPI] = 10^{-5}M (dark yellow) to 7.5 x 10^{-4}M (red). Inset spectra concentration-dependent transition of the intensity of the CD signal of DAPI gel monitored at 486 nm. Linear increase of CD intensity suggests the enhancement of handedness of the coiled nanofibers formed during self-assembly process.

11) Temperature dependent CD results of DAPI systems

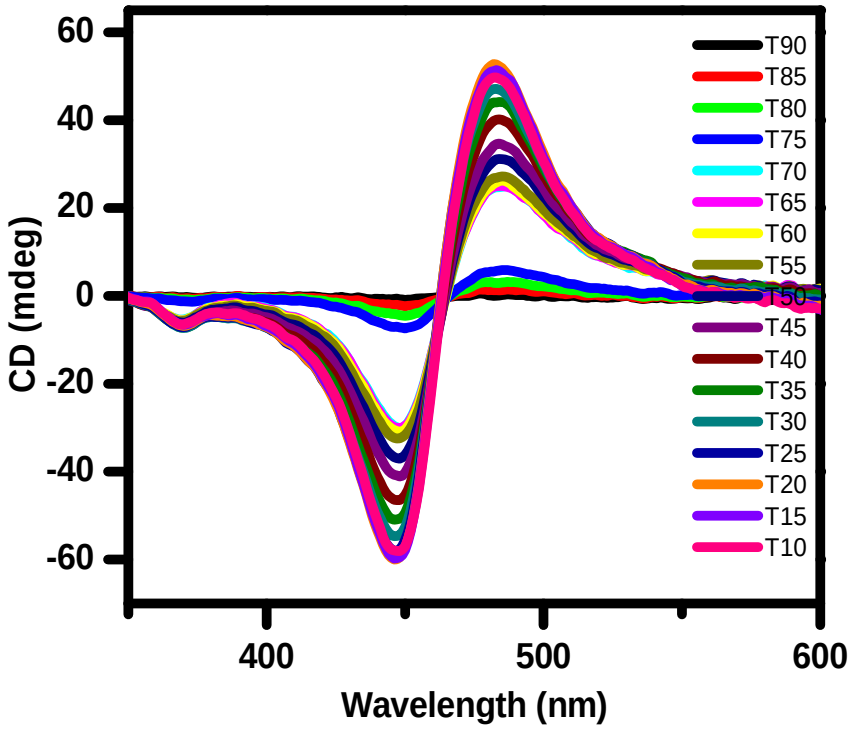


Fig. S8 Temperature dependent CD spectra of DAPI gel (5 x 10^{-4} M) using 1.00 mm quartz cell. .

12) DSC plot of LAPI gel

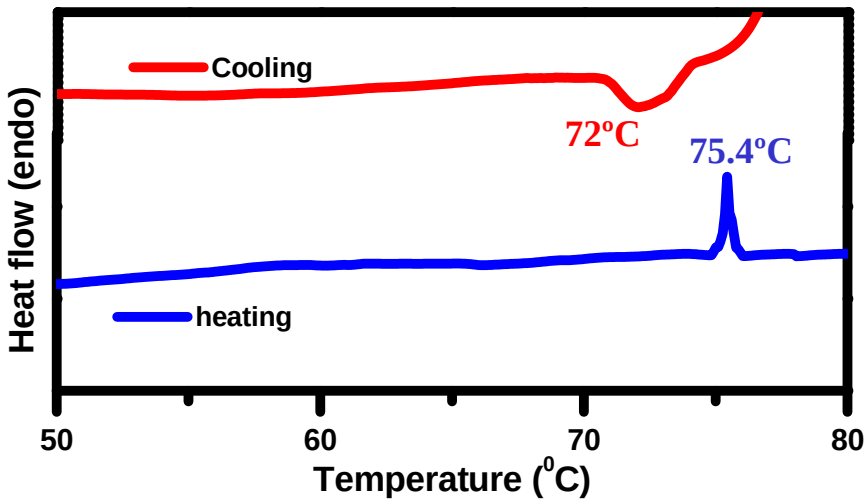


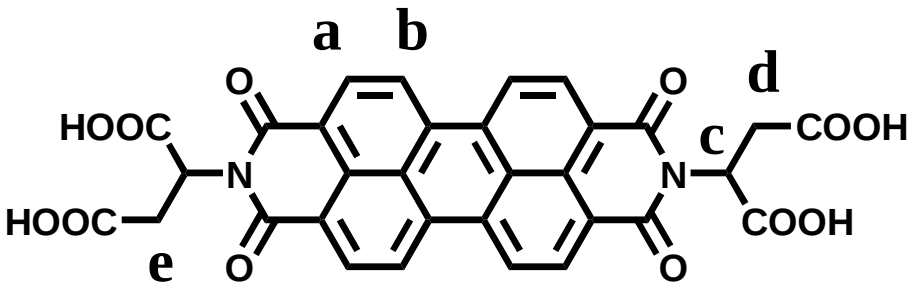
Fig. S9: Typical DSC thermograms of LAPI (0.6% w/v) hydrogel at heating rate 10°C/min and cooling rate 5°C/min. The thermal results indicate the thermo-reversibility of LAPI hydrogel.

13) NMR studies

^{1}H NMR spectroscopy of API solution and API gel:

Initially ^{1}H NMR spectra of API solution having concentration of 5 x 10^{-3} M was taken in a 300 MHz instrument at 25^0C. Then prepared the solution of APIG with same concentration in another tube and add 50 µl of 0.2 N of DCl in D_2O to lower the pH of the solution. The whole solution transformed to gel after waiting for overnight. Then its spectra were taken in the same instrument at same temperature.

Sl no.	Types of protons	δ value (ppm), API at 25^0C under 300 MHz	δ value (ppm), APIG at 25^0C under 300 MHz
1.	a	8.17	7.21
2.	b	7.92	6.17
2.	c	5.84	5.63
4.	d	3.15	3.20
5.	e	2.94	2.89



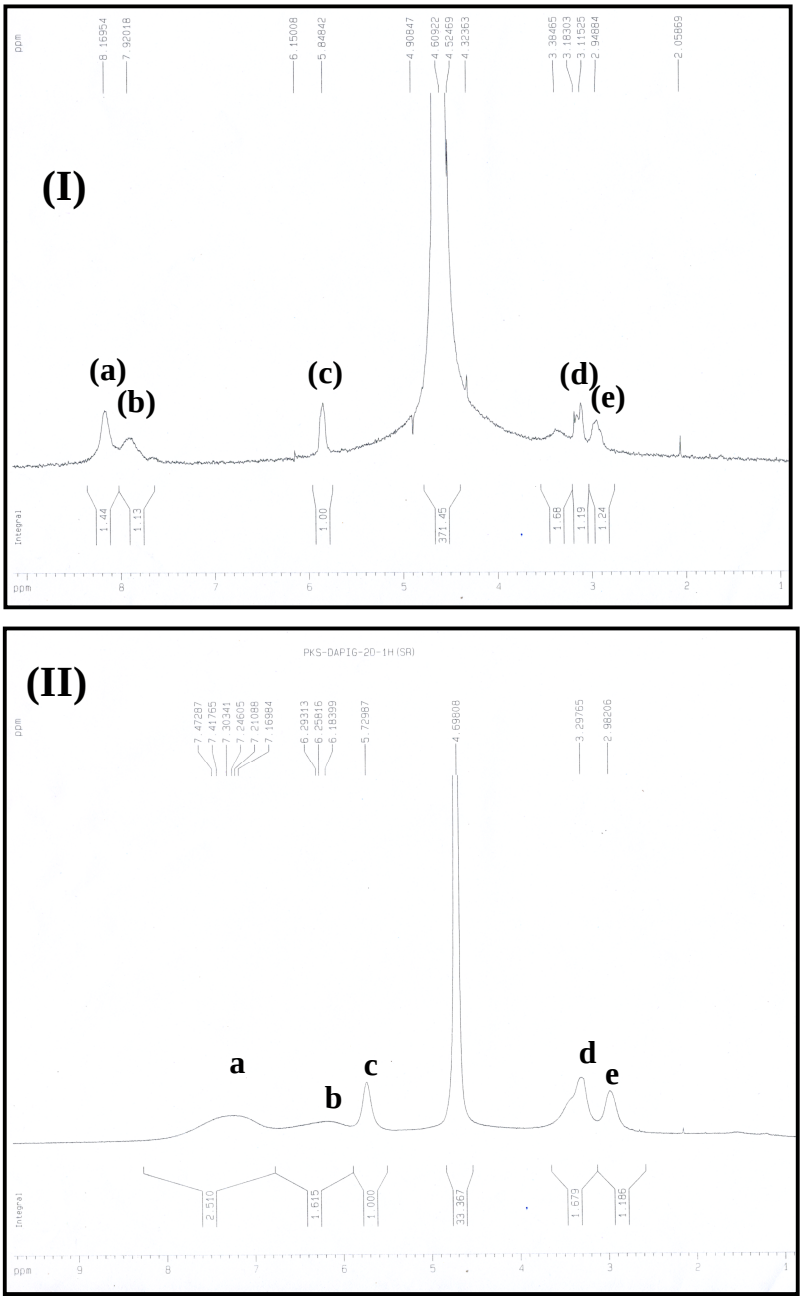


Fig. S10: ^{1}H NMR of API solution (I), and hydrogel (II) at room temperature in D_2O under 300 MHz Bruker instrument. NMR investigation clearly reveals the broadening of the proton signal of perylene core in the gel state with upfield shifting, surmising greater shielding of these protons during aggregation.[4]

14) XRD results

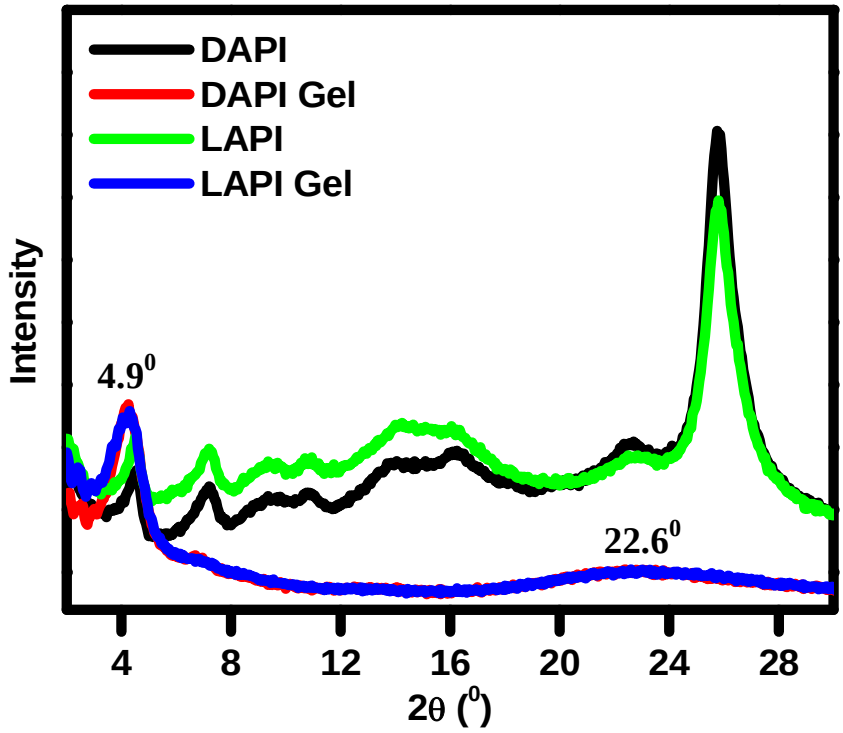


Fig. S11 X-ray diffraction of LAPI, DAPI, LAPI gel and DAPI gel dried gel. In X-ray diffraction (XRD) powder patterns, interestingly, it shows a broad spectrum for dried gel with a characteristic band at 1.80 nm and at 0.39 nm for π-π stacking. The presence of diffraction peak around 22.6° in x–ray diffraction of the dried gel has indicated a well-defined, repeatative and compact π–π stacking of perylene cores separated by 3.9 Å in gel fiber.

15) FTIR Results

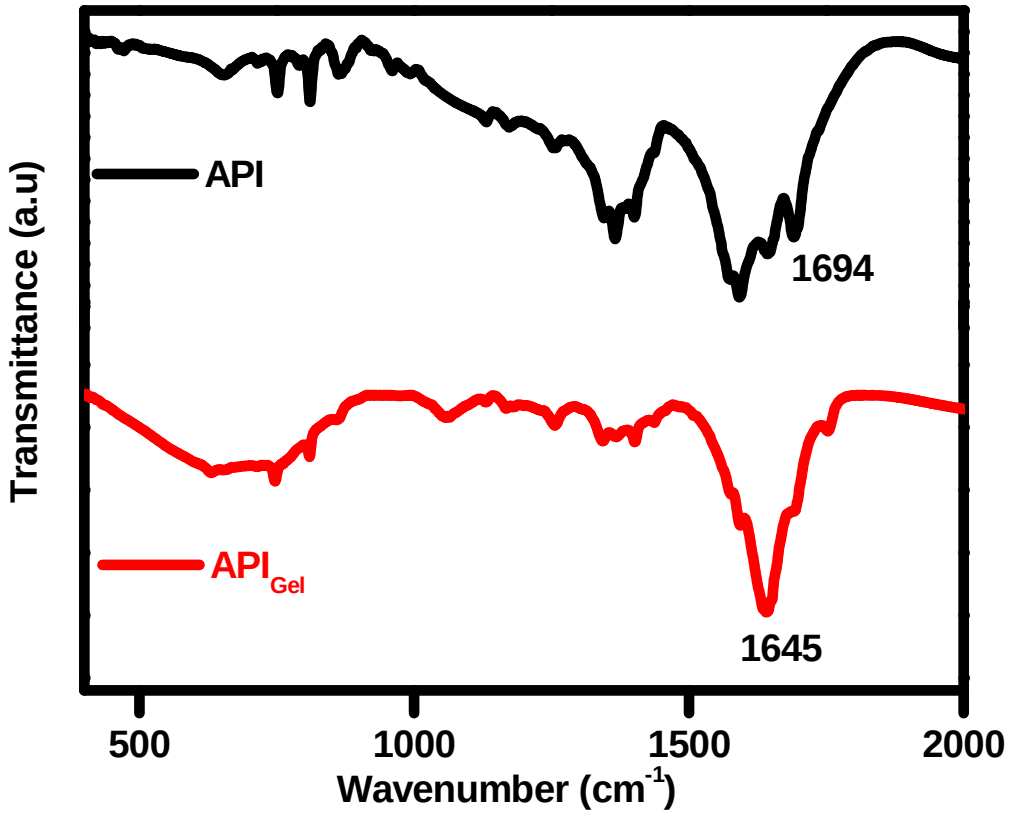


Fig. S12 FT-IR diluted with KBr for pure API derivative (top) and API dried gel (bottom).

It clearly reveals the shifting of acid carbonyl peak towards lower wave number by 49 cm-1 which obviously due to formation of strong intermolecular hydrogen bonding during gelation.

16) Cell Vibility Assays

To evaluate the effect of LAPI on cell vibility, neuronal survival assay was performed. For this study the SH-SY5Y cells were seeded in Dulbecco's modified Eagle's high-glucose medium with 10% fetal bovine serum, 100 units/ml penicillin G and 100 µg/ml streptomycin (Gibco, USA) on cleaned and sterile round cover slides in 24 well plates at a density of 10,000 cells per well for 24 hours in a 5% CO_2 humidified environment at 37°C. After 24 hours of incubation the media was discarded and aqueous solution of LAPI was added to cells at a final concentration of 50 µM in media and cells were further incubated. After 24 hours of incubation, the cellular viability was assayed using Calcein-AM fluoresenct live/dead stain (Invitrogen, USA). In breif, ready to use solution (1 mg/ml) of Calcein-AM was added to 24 wells at a final concentration of 1 µg/ml and after 10 minutes of incubation in dark, the cells were imaged under confocal microscope (Olympus IX81). Around five different areas of three independent samples were imaged at room temperature. Further to evaluate the dose dependent effect of LAPI on cell survival, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay[5] was performed. MTT assay is a well established routine assay for evaluating the cellular toxicity of any compound. For MTT assay the cells were seeded as described above in a 96 well tissue culture plate. After 24 hours of incubation the media was discarded and LAPI was added to cells. The final concentrations of LAPI were 25 µM, 50 µM and 100 µM in media. Cells were incubated for 24 hours in a 5% CO_2 humidified environment at 37°C. After 24 hours 10 µl of MTT (5 mg/ml in PBS) was added to each wells and incubated for 4 hours. After 4 hours, 100 µl of a solution containing 50 % dimethylformamide and 20 % SDS (pH 4.8) was added to each wells and incubated overnight. After overnight incubation absorption values at 560 nm were determined with an automatic microtiter plate reader (Thermo Fisher Scientific).

17) LAPI cellular internalization and imaging

To study the LAPI internalization in neuronal cells, SH-SY5Y cells were seeded in 24 well tissue culture flasks, as described earlier. Aqueous solution of LAPI was diluted in cell culture media to cells at a final concentration of 20 μM. After 24 hours of incubation of SH-SY5Y neurons with LAPI, cells were gently washed with PBS to remove the excess LAPI. Cells were fixed with 3.5% formaldehyde at 37°C for 30 minutes. After fixing, the cells on cover slide were mounted on clean glass slide (Himedia, India) with mounting media -fluoroshield with 4′,6-Diamidino-2-phenylindole dihydrochloride (Sigma, USA) and sealed with nail polish. Cells were imaged under confocal microscope (Olympus IX81) using red and blue filters.

For confocal lamda scan study, similar experiments were performed. LAPI was added to cells at a final concentration of 1 μM and 50 μM after diluting in cell culture media. The cells were further incubated for 4 hours. After the incubation the media containing LAPI was discarded and cells were washed with PBS to remove excess unbound dye. The cells were fixed and mounted on a cleaned glass slides as described above. The cells were imaged under confocal microscope (Carl Zeiss LSM 510 Meta) using lamda scan mode. In brief, the samples were excited at 488 nm and images were acquired in the emission range of 500-750 nm. Different areas of the acquired image were selected manually and the corresponding emission spectra were processed using ZEN Imaging software (Carl Zeiss Micro Imaging GmbH, Germany).

18) References

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