Electronic Supplementary Information (ESI)

Perfluoroarene induces a pentapeptidic hydrotrope into a pH tolerant hydrogel allowing naked eye sensing of Ca^{2+} ions

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Physical Measurements and Instrumentation.

Materials and Methods: All chemicals and solvents were obtained from well-known commercial sources which were used without further purification. ^{1}H-NMR and ^{13}C-NMR spectra were recorded in Bruker-400 Advance NMR spectrometer. Mass spectrometry of individual compound was performed using a MicroMass ESI-TOF MS instrument.

Gelation Studies. The tube inversion method was used to confirm the gel formation. If a gel was formed, it was evaluated quantitatively by determining the critical gelator concentration (CGC), which is the minimum amount of the gelator required to immobilize 1 mL of solvent.

FT-IR Spectroscopy. Solution of specified compounds was drop-coated and the spectrum of the resulting material was recorded in Perkin Elmer Spectrum BX FT-IR system.

UV-Vis absorption and Fluorescence Spectroscopy. The UV-Vis and fluorescence spectra of specified solutions were recorded on Shimadzu model 2100 spectrophotometer and a Hitachi F-4500 spectrofluorimeter respectively, both equipped with a temperature controller bath.

Circular Dichroism Spectroscopy. All the CD spectra of specified solutions were recorded on a JASCO instrument (Model J-815-150S). Experiments were conducted by purging dry N_2 gas continuously. Data were collected in a quartz cuvette of 1 mm path length between 200 to 500 nm.

Atomic Force Microscopy (AFM). Solutions of the aggregates were drop-cast on freshly cleaved mica strips and then carefully air-dried. Each of the samples was analyzed using a JPK 00901 instrument and Nano-Wizard software: Tapping mode.

Transmission Electron Microscopy (TEM). Solutions of the aggregates at specified concentration were drop-coated on carbon-coated copper grids and then carefully air-dried. The TEM images were taken at an accelerating voltage of 200 kV using a TECNAI F30 instrument.

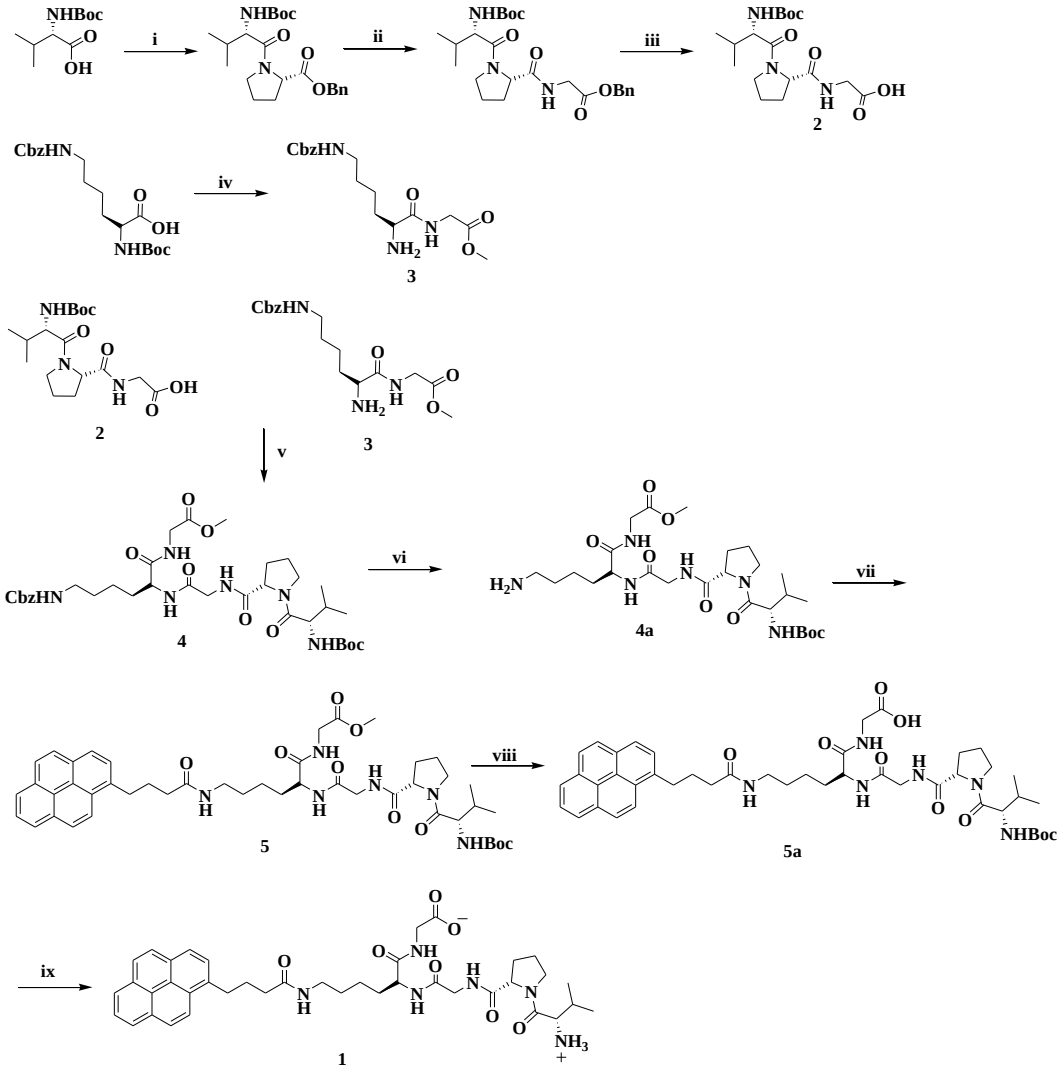
Dynamic Light Scattering (DLS) and Zeta Potential. DLS measurements were performed at room temperature using a Malvern Zetasizer Nano ZS particle sizer (Malvern Instruments Inc., Westborough, MA). Samples were prepared and examined under dust-free conditions. Zeta potential was recorded in the same instrument by zeta dip cell.

Differential Scanning Calorimetry (DSC). Solution phase DSC experiment of PyP (3 mM) was performed using a CSC-4100 multicell differential scanning calorimeter (Calorimetric Sciences Corporation, Utah, USA) with a fixed heating and cooling rate at 10 °C per hour.

Rheological Studies. For rheology experiment of the gels, an Anton Paar 100 rheometer with a cone and plate geometry (CP 25-2) and Peltier temperature controlling system were used. The gap distance between the cone and the plate was fixed at 0.05 mm for all experiments. The gels were scooped on the plate of the rheometer. For an oscillatory strain amplitude sweep experiment, the oscillation frequency was kept constant at 1 Hz and the applied strain was varied in the range of 0.001-300 % at 20 °C. US-200 software converted the torque measurements into G' (the storage modulus) and G'' (the loss modulus). Oscillatory frequency sweep experiments were conducted in the linear viscoelastic region (strain 0.01%).

X-ray Diffraction. The gel of 1:1 PyP:OFN was inserted in a capillary and slowly freeze-dried under vacuum for XRD measurement. X-ray diffraction was recorded in EMPYREAN (PANalytical) instrument from 0.9-30° (2θ) with a scan rate 0.5° per minute and step size 0.02°. The X-ray beam, generated by Cu anode at the wavelength of 1.5418Å was used. The Bragg's equation was used to analyze the data.

Scheme 1[a]



[a]**Reagents, Conditions and Yields:** i) DIC, DCM, HOBt, DIPEA, L-Pro-OBn. HCl, rt, 12 h, 70%; ii) a. H_2, 10% Pd-C, EtOH, 10 h, 2atm, 90%; b. DIC, DCM, HOBt, Gly-OBn. HCl, DIPEA, 12 h, rt, 75%; iii) H_2, 10% Pd-C, EtOH, 10h, 2 atm, 90%; iv) a. Gly-OMe. HCl, DIC, DCM, HOBt, DIPEA, 12h, rt, 80%; b. DCM : TFA (3:1), 1.5 h, 90%; v) DIC, DCM, HOBt, DIPEA, 10 h, rt, 75%; vi) H_2, 10% Pd-C, EtOH, 10 h, 2 atm, rt; vii) 1-Pyrenebutyric acid, DIC, DCM, HOBt, DIPEA, 12 h, rt, after two steps 60%; viii) LiOH.H_2O, THF : H_2O (3:1), 1.5 h, rt; ix) DCM : TFA (3:1), 1.5 h, rt, after two steps 70%.

Synthetic procedure and characterization: Boc-Val-Pro-Gly-OH (2) and NH_2-Lys(Z)-Gly-OMe (3) were synthesized according to the reported procedure in literature.[S1,S2]

Boc-Val-Pro-Gly-Lys(Z)-Gly-OMe (4): To a solution of compound 2 (2 g, 3.5 mmol) in 20 mL dichloromethane, DIC (0.53 g, 4.2 mmol) and HOBt (0.57 g, 4.22 mmol) were added. The mixture was stirred at 0 °C for 30 minute. After that Compound 3 (1.23 g, 3.5 mmol) and DIPEA (0.45 g, 3.5 mmol) were added to it and stirred at room temperature for 10 h. Then solvent was removed from reaction mixture in vacuum and diluted with 150 mL ethyl acetate and washed with water (2 X 30 mL) and brine (2 X 30 mL). Ethyl acetate layer was dried over anhydrous sodium sulfate and evaporated in vacuum to get the crude product. The compound was purified by column chromatography with chloroform and methanol (v/v 100:4) as solvent. Yield = 2.46 g, 75%. FT-IR (Neat, cm^{-1}) 3696, 3060, 2961, 2934, 2870, 1788, 1753, 1661, 1528, 1447, 1406, 1367, 1247, 1168, 1093, 1016; ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 0.94-0.99 (m, 6H), 1.41 (s, 11H), 1.49-1.52 (m, 2H), 1.86-1.99 (m, 3H), 2.01-2.17 (m, 3H), 3.17-3.18 (d, 2H), 3.48-3.49 (d, 1H), 3.61-3.64 (m, 2H), 3.17 (s, 3H), 3.84-3.90 (m, 2H), 4.11-4.32 (m, 4H), 4.50-4.52 (d, 1H), 5.08 (s, 3H), 5.43-5.45 (d, 1H), 7.30-7.34 (m, 5H), 7.39-7.41 (d, 1H), 7.44 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 18.2, 19.1, 22.4, 25.3, 28.3, 28.9, 29.0, 31.1, 31.5, 40.4, 40.9, 43.3, 47.9, 52.3, 53.6, 57.6, 61.2, 66.4, 79.6, 127.9, 128.0, 128.4, 136.6, 155.8, 156.5, 169.5, 170.9, 172.3, 172.4; ESI-MS (HRMS) Calcd for $[C_{34}H_{52}N_6O_{10} + Na]^+$ 727.3643; found: 727.3644.

Boc-Val-Pro-Gly-Lys(NHPy)-Gly-OMe (5): Compound 4 (1.5 g, 2.02 mmol) was dissolved in 20 mL ethanol and 10% Pd-C (0.1 g) was added to it. Solution was stirred under H_2 at 2 atmosphere pressure for 6 h. Then reaction mixture was passed through celite pad to remove palladium and charcoal. Ethanol was removed in vacuum to get product (4a) which was used without further purification. To a solution of 1-pyrenebutyric acid (0.63 g, 2.2 mmol) in 20 mL dichloromethane, DIC (0.277, 2.2 mmol) and HOBt (0.297, 2.2 mmol) were added. The mixture was stirred for 30 minute at 0 °C. Compound (4a) from the previous step and DIPEA (0.26 g, 2.02 mmol) were then added to it. The reaction mixture was stirred for additional 10 h at room temperature. After that organic solvent was evaporated in vacuum. The solid crude was purified by column chromatography with chloroform and methanol (v/v 100:4) as solvent. Yield = 1.074 g, 60% after two steps. FT-IR (Neat, cm^{-1}) 3782, 327, 3696, 3045, 2959, 2928, 2861, 1750, 1647, 1530, 1443, 1407, 1367, 1217, 1165, 1108, 1016; ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 0.94-0.99 (m, 6H), 1.3-1.5 (m, 7H), 1.38 (s, 9H), 1.58-1.63 (m, 1H), 1.82-1.99 (m, 7H), 2.14-2.15 (d, 2H), 2.23-2.25 (d, 2H), 3.13-3.18 (m, 3H), 3.31 (m, 2H), 3.53-3.68 (m, 3H), 3.61 (s, 3H), 3.79-3.85 (m, 2H), 4.01-4.06 (m, 1H), 4.12-4.22 (m, 3H), 4.47-4.48 (d, 1H), 5.54-5.57 (d, 1H), 6.22-6.24 (d, 1H), 7.4 (1H), 7.57-7.59 (d, 1H), 7.79-7.80 (d, 2H), 7.92-7.96 (m, 3H), 8.03-8.12 (m, 4H), 8.23-8.25 (d, 1H). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 18.1, 19.0, 22.5, 25.1, 27.3, 28.2, 28.3, 28.8, 29.5, 31.0, 31.5, 32.6, 35.8, 38.7, 40.8, 43.1, 47.8, 52.1, 53.4, 57.4, 61.0, 79.5, 123.3, 124.6, 124.7, 124.8, 124.9, 125.7, 126.5, 127.2, 127.3, 128.5, 129.7, 130.7, 131.2, 135.9, 155.7, 169.6, 170.7, 172.2, 172.5, 172.9; ESI-MS (HRMS) Calcd for $[C_{46}H_{60}N_6O_9 + Na]^+$ 863.4319; found: 863.4316.

$^+NH_3$-Val-Pro-Gly-Lys(NHPy)-Gly-O^- (1): Compound 5 (1.074 g, 1.278 mmol) was dissolved in 15 mL THF and water (v/v 3:1) and lithium hydroxide monohydrate (0.08 g, 1.917 mmol) was added to it at 0 °C. The reaction mixture was stirred at room temperature for 1.5 h. Then solvent was removed and diluted with chloroform. The chloroform layer was washed with water to remove excess lithium hydroxide and dried over anhydrous sodium sulfate. Chloroform was removed to in vacuum to get the product 5a. The product (5a) was used for the next step without further purification. Compound 5a was dissolved in 12 mL of CH_2Cl_2: TFA (3:1) at 0 °C and stirred at room temperature for 1.5 h. After completion of the reaction, the solvent was removed, the product was neutralized with aqueous sodium bicarbonate. Compound was extracted with chloroform and methanol (v/v 100:2). The solvent was evaporated to get the pure product 1. Yield = 0.8 g, 70% after two steps. Compound was hygroscopic. FT-IR (Neat, cm^{-1}) 3618, 3299, 3086, 2937, 2869, 1670, 1538, 1442, 1373, 1247, 1202, 1136, 1083, 1014; ^{1}H NMR (400 MHz, CD_3OD, ppm): δ 0.98-1.05 (m, 6H), 1.37 (m, 2H), 1.49-1.50 (d, 2H), 1.67 (m, 1H), 1.89 (m, 3H), 2.03 (1H), 2.13-2.16 (m, 4H), 2.34 (t, 2H, J = 7.2), 3.17 (2H), 3.34 (m, 4H), 3.49-3.51 (d, 1H), 3.65-3.69 (m, 2H), 3.84 (2H), 3.59-4.04 (m, 2H), 4.35 (1H), 4.48 (1H), 7.88-7.89 (d, 1H), 7.97-8.03 (m, 3H), 8.11-8.19 (m, 4H), 8.31-8.34 (d, 1H). 13C NMR (100 MHz, CD_3OD, ppm): δ 17.68, 19.03, 23.96, 26.08, 29.00, 29.66, 30.32, 31.01, 32.58, 33.66, 36.77, 40.04, 43.61, 54.44, 58.24, 57.94, 62.26, 124.21, 125.67, 125.79, 125.88, 125.98, 126.84, 127.51, 128.19, 128.29, 128.37, 129.68, 131.09, 132.06, 132.57, 137.57, 162.77, 163.12, 171.54, 174.18, 174.31, 175.69. ESI-MS (HRMS) Calcd for $[C_{40}H_{50}N_6O_7 + H]^+$: 727.3819; found: 727.3822. Anal. Calcd for $C_{40}H_{50}N_6O_7.3H_2O$: C 61.52, H 7.23, N 10.76. Found: C 61.48, H 7.26, N 10.81

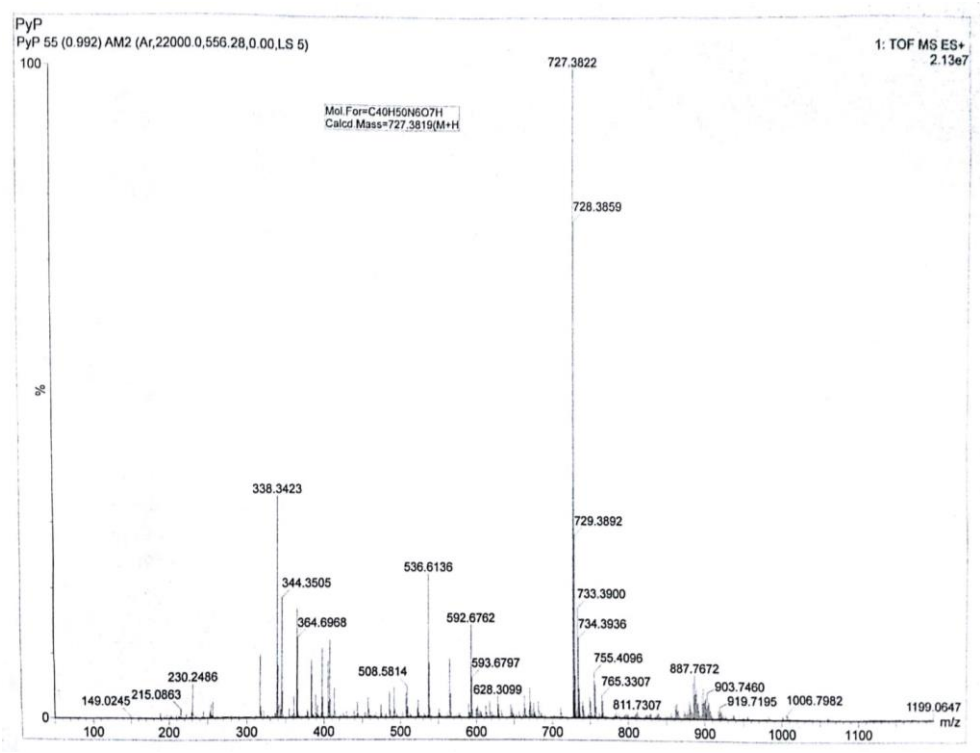
References:

S1. F-C. Wu; C-S. Da; Z-X. Du; Q-P. Guo, W-E. Li, L. Yi; Y-N. Jia; J. Ma. *J. Org. Chem.* 2009, **74**, 4812.

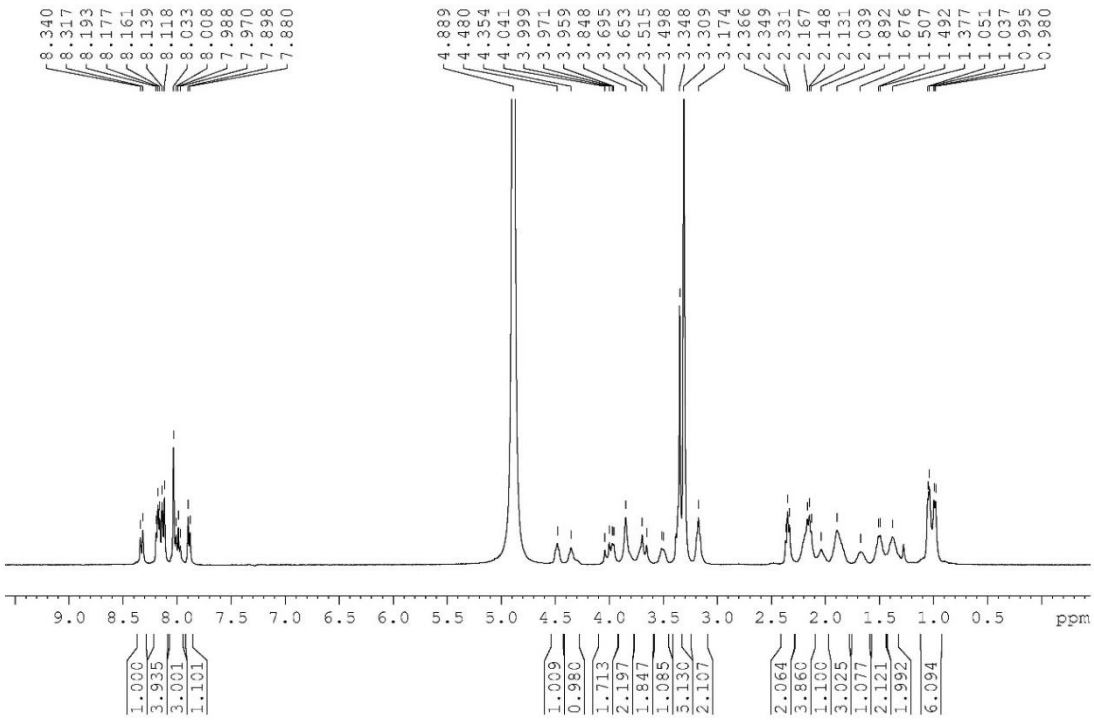
S2. P. D. Bailey and G. A. Crofts. *Tetrahedron letters* 1992, **33**, 3207.

Characterization of PyP (1).

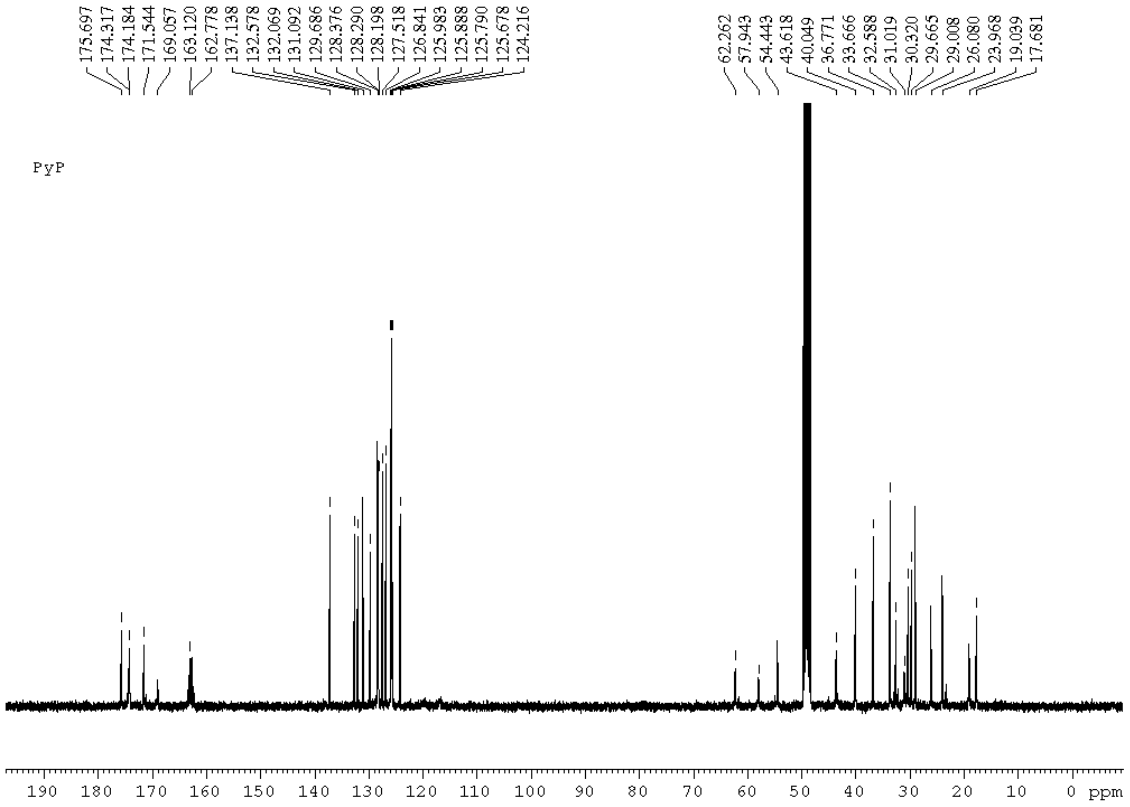
Mass spectrum



^{1}H NMR



^{13}C NMR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128.290
128.198
127.518
126.841
125.983
125.888
125.790
125.678
124.216
62.262
57.943
54.443
43.618
40.049
36.771
33.666
32.588
31.019
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17.681
PyP
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

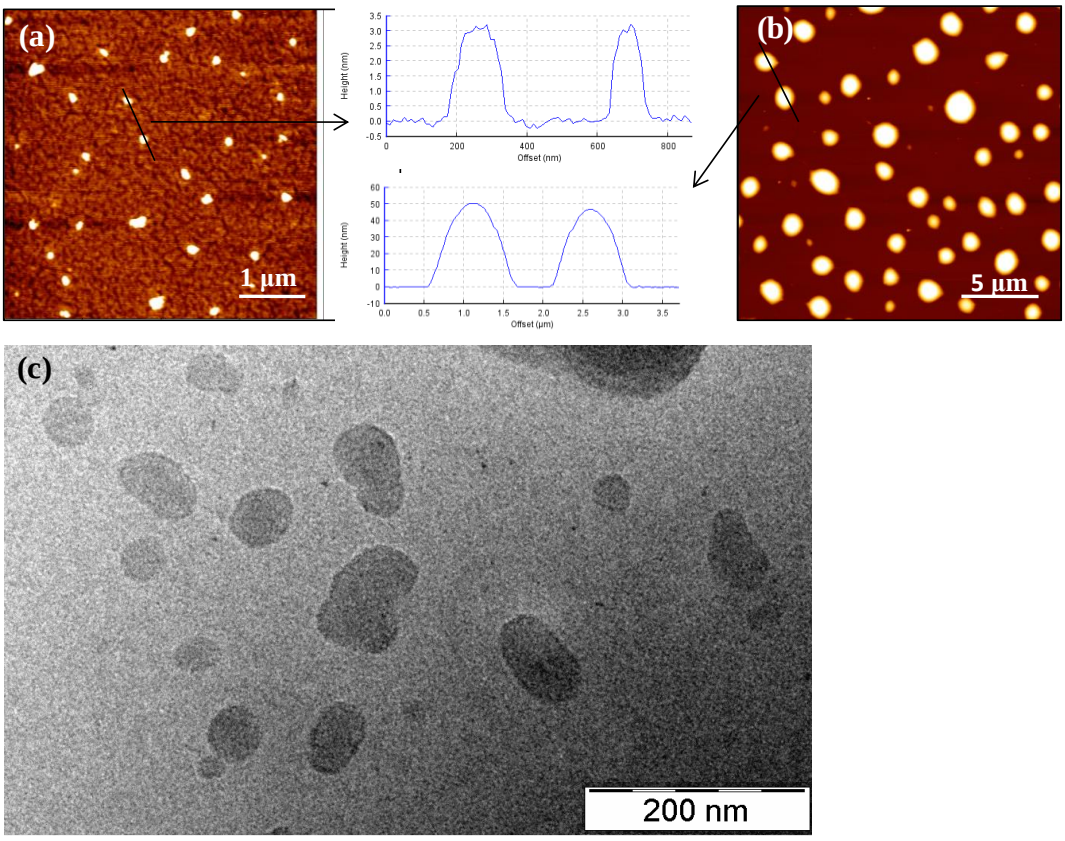

Fig. S1. (a, b) AFM images of the solution of PyP (~0.15 mM) in pure water drop-coated on mica below and above the phase transition temperature and their height images respectively. (c) TEM image of the solution of PyP (~0.15 mM) in pure water drop-coated on the carbon-coated copper grid.

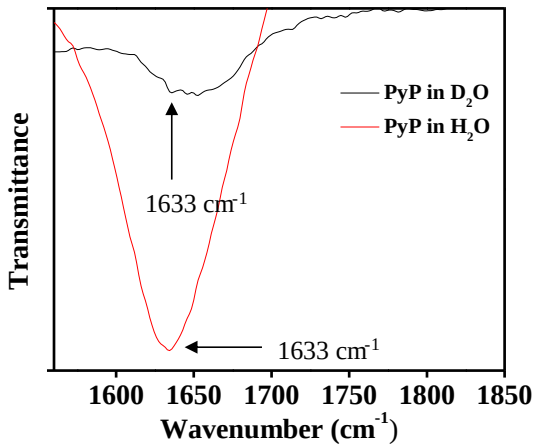

Fig. S2. Selected region of FT-IR spectra of PyP in D_2O and H_2O.

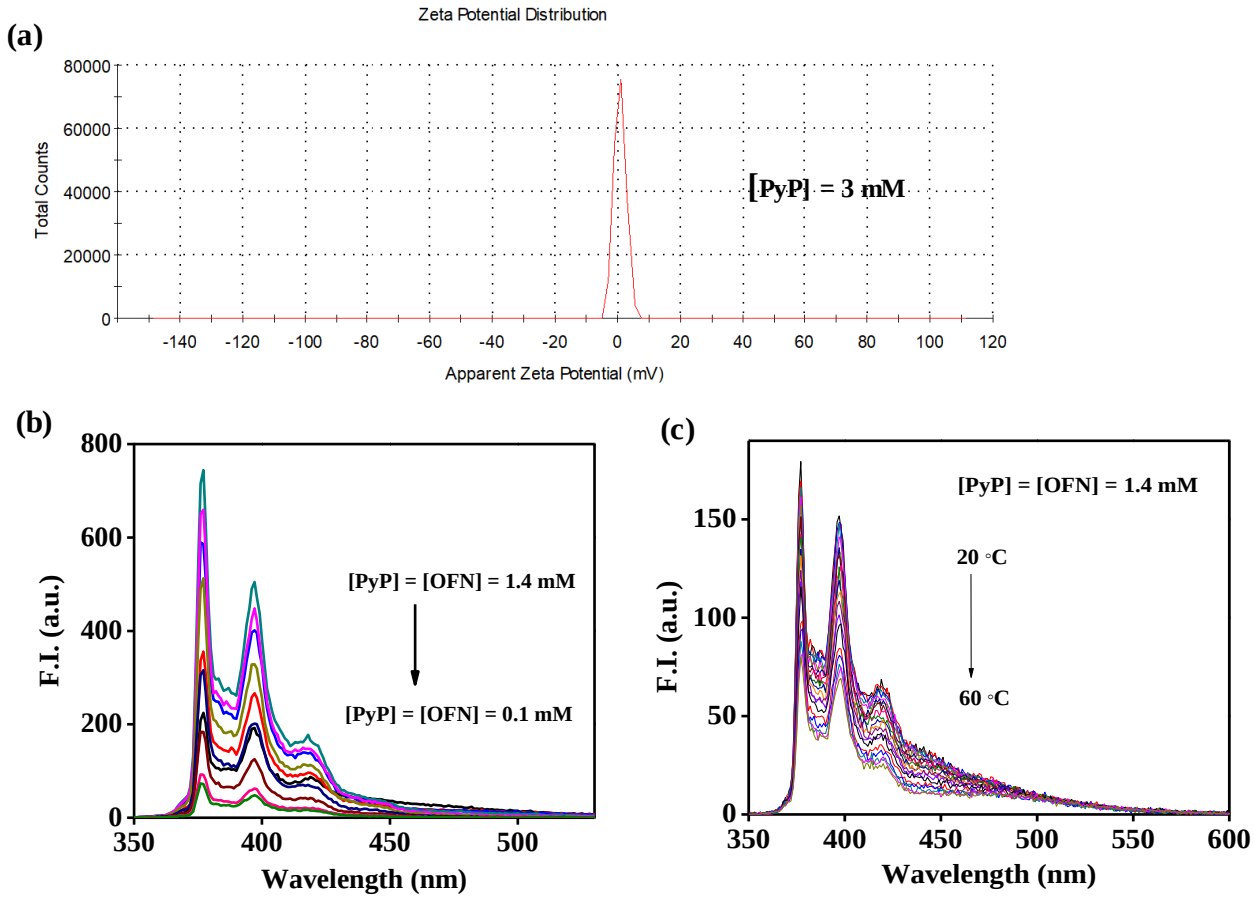

Fig. S3. (a) Zeta potential of PyP in water, (b) Concentration dependent emission spectra of 1:1 PyP:OFN in EtOH:H_2O = 2:7 v/v, (c) Temperature-dependent emission spectra of PyP-OFN in EtOH:H_2O = 2:7 v/v.

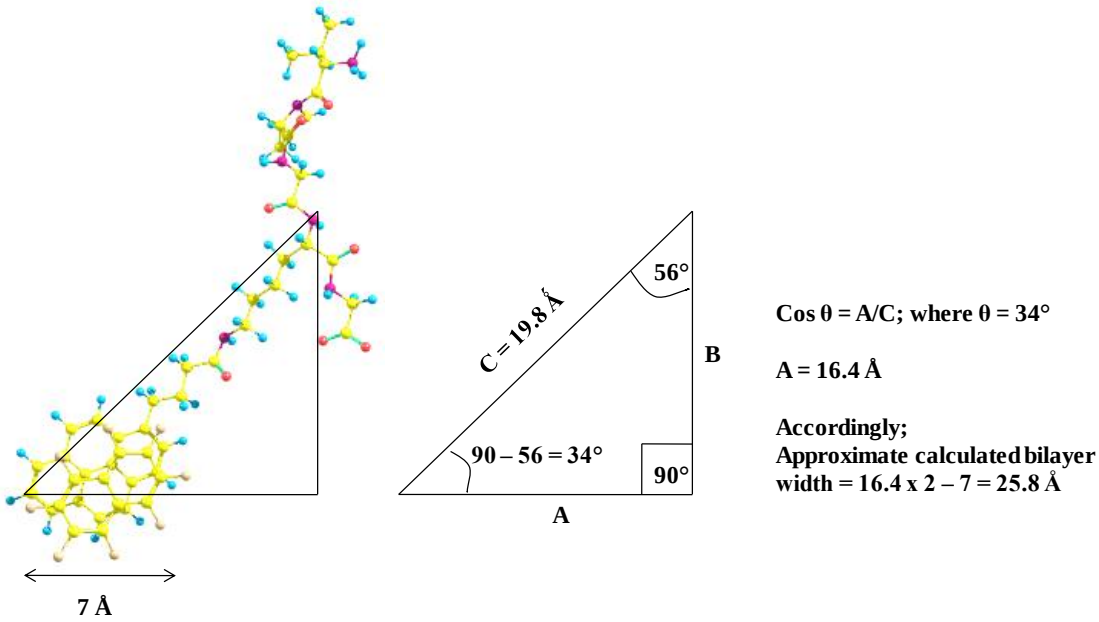

Fig. S4. Energy minimized conformation of 1:1 PyP:OFN unit as obtained using B3LYP/6-31G* level of computations (left). Based on this energy-minimized structure of the gelator unit, approximate calculated bilayer width has been determined to be 25.8 Å (right).

Table S1. Summary of addition of different metal ions to the solution of the native gelator; [PyP] = [OFN] = 2.2 mM.

Metal ions	**Visual change in the solution of the native gelator ; [PyP] = [OFN] = 2.2 mM**
K^+	No change
Ni^{2+}	No change
Mg^{2+}	Precipitate
Co^{2+}	Milky solution
Zn^{2+}	Precipitate
Ca^{2+}	Gel
Na^+	No change
Cu^{2+}	No change
Fe^{3+}	No change
Fe^{2+}	No change
Hg^{2+}	No change
Pb^{2+}	No change

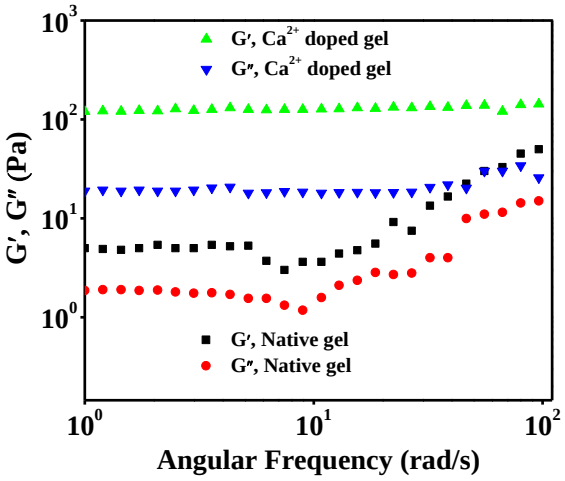

Fig. S5**.** Oscillatory frequency sweep experiments of the native and Ca^{2+} doped gel of 1:1 PyP:OFN ([PyP] = 10 mM) in EtOH:H_2O = 2:7 v/v respectively.

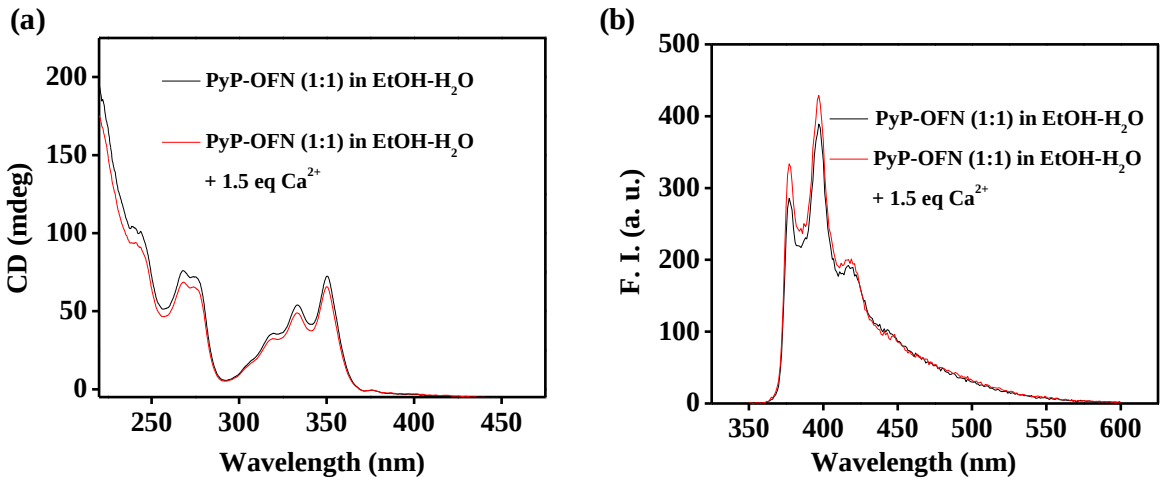

Fig. S6. Changes in (a) fluorescence emission and (b) CD spectra of PyP-OFN (1:1) in EtOH-H_2O = 2:7 v/v after addition of Ca^{2+} ion. Concentration of PyP for fluorescence and CD measurements were 1 mg/mL and 0.8 mg/mL respectively.

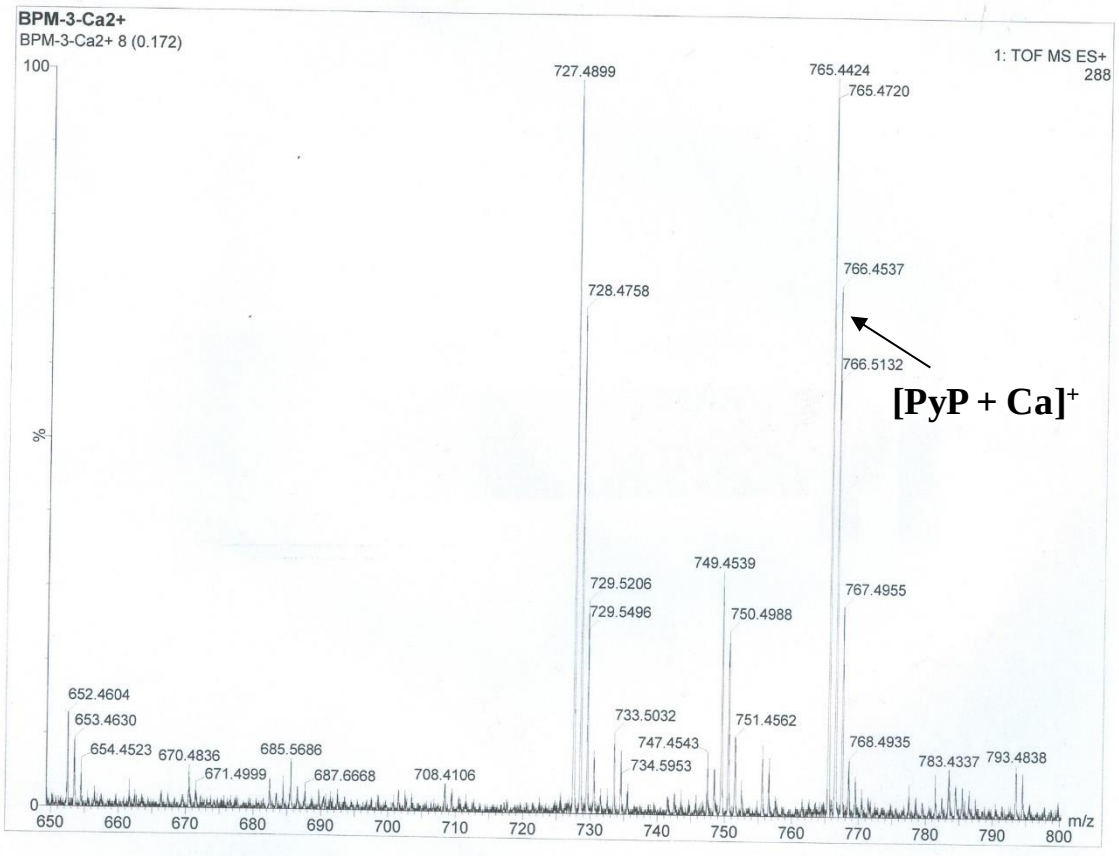

Fig. S7. Mass spectrum of Ca^{2+} doped gel.