Electronic Supplementary Material (ESI) for Journal of Materials Chemistry A.

Phthalate Mediated Hydrogelation of a Pyrene based System: A Novel Scaffold for Shape-Persistent, Self-Healing Luminescent Soft Material

Subham Bhattacharjee,[a] and Santanu Bhattacharya*[a, b]

[a]Department of Organic Chemistry, Indian Institute of Science, Bangalore, India. Fax: +91-80-23600529; Tel: +91-80-22932664; E-mail: sb@orgchem.iisc.ernet.in

[b]Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, India. Fax: +91-80-23600529; Tel: +91-80-22932664; E-mail: sb@orgchem.iisc.ernet.in

Table of contents:

1. Physical Measurements and Instrumentation.

2. Synthesis.

3. Figure S1: Concentration dependence of the gel melting temperature (T_{gel}).

4. Table S1: Summary of pK_a values of different carboxylic acids and their salt formation and gel formation ability with PyHis in water.

5. Figure S2: IR spectra of PyHis alone, PyHis:PA = 1:1 hydrogel and Eu^{3+} doped PyHis:PA = 1:1 hydrogel; Comparision of the IR spectra of PyHis:PA = 1:1 system with PyHis alone as well as PyHis:IPA = 1:1 and PyHis:TPA = 1:1; Concentration dependent emission spectra of the PyHis:PA = 1:1 hydrogel; Emission spectra and the corresponding photographs of the solution of Py-His alone and its equimolar mixture with PA and IPA and TPA respectively from 9:1 H_2O/MeOH mixture.

6. Figure S3: Concentration dependent CD spectra of the PyHis:PA = 1:1 hydrogel.

7. Figure S4: SEM images of the PyHis:PA = 1:1 system in water; [PyHis] = 0.4 mM. Fluorescence microscopic images of the PyHis:PA = 1:1 system, [PyHis] = 0.4 mM. Careful analysis of the AFM image showed the presence of multiple spherical aggregates along with the coiled-coil left-handed helical fibers and height images of two of such spherical aggregates were shown for clarity; DLS measurements of a solution of PyHis:PA = 1:1 system shows the average hydrodynamic diameter (D_h) of 276 ± 15 nm, [PyHis] = 0.2 mM.

8. Figure S5: Variable temperature ^{1}H-NMR spectra of the PyHis:PA = 1:1 hydrogel

9. Figure S6. X-ray diffraction studies of the PyHis:PA = 1:1 hydrogel.

10. Figure S7. Energy minimized conformation of PyHis:PA = 1:1 salt as obtained using B3LYP/6-31G* level of computations.

11. Figure S8: Oscillatory amplitude sweep and frequency sweep experiments of PyHis:PA = 1:1 hydrogel at three different concentrations respectively; oscillatory frequency sweep experiments of the native gel and Eu^{3+} doped hydrogel.

12. Figure S9: Stress-strain curve and creep-recovery experiment at different stress levels of the PyHis:PA = 1:1 hydrogel.

1. Physical Measurements and Instrumentation.

Gelation Studies. The detail procedure for hydrogelation was discussed in the main paper. The formation of the gel was confirmed using tube inversion method. If a gel was formed, it was evaluated quantitatively by determining the critical gelator concentration (CGC), which is the minimum amount of gelator required to immobilize 1 mL of water. Gelation test including evaluation of CGC was carried out in test tubes of 10x75 mm dimensions. For evaluation of CGC, a known amount of PyHis:PA = 1:1 mixture in pure water was heated to 75-80 °C, which resulted in a clear solution. The resulting clear solution on spontaneous cooling transformed into optically transparent, robust hydrogel within 1-2 min. The hydrogelation propensity of the PyHis:PA = 1:1 mixture has been checked systematically by gradually lowering the amount of the gelators. In that way the minimum concentration of the gelators that required to immobilize 1 mL of water, i.e, CGC value was determined as reported with other systems. The above measurement was carried out three times and the average CGC value has been reported.

The gelators with a CGC value of ≤ 0.1 wt% have been reported to belong to the category of supergelators.[1] Since the total amount required (0.74 mg of PyHis + 0.28 mg of PA = 1.02 mg) to rigidify 1 mL of water is 0.1 wt%, i.e, 1 mg/mL, the present gelator has been categorized as a supergelator.

1. S. Dong, B. Zheng, D. Xu, X. Yan, M. Zhang and F. Huang, *Adv. Mater.* 2012, **24**, 3191.

FT-IR Spectroscopy. Solution of specified compounds or their designated mixtures with PA at 1:1 ratio were drop-cast on the CaF_2 cell and dried under vacuum and the spectra of the resulting material was recorded in Perkin Elmer Spectrum BX FT-IR system.

^{1}H-NMR Spectroscopic Studies of the Hydrogel. For the variable temperature ^{1}H-NMR spectroscopic study, the PyHis:PA = 1:1 hydrogel ([PyHis] = 9.7 mM) in D_2O was prepared. The hot sol was then introduced into a NMR tube. The resultant hot sol in the NMR tube was left to cool to form the hydrogel again, which was gradually heated and NMR signals were recorded at various temperatures.

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 performed by purging dry N_2 gas continuously. Data were collected in a quartz cuvette of 1 mm path length between 200 to 500 nm.

Scanning Electron Microscopy (SEM). The gels or solutions of the aggregates of specified mixtures were carefully drop-cast onto the brass stubs and were allowed to air-dry overnight. The samples were then coated with gold vapor and analyzed on a Quanta 200 SEM operated at 10-15 kV.

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 were analyzed using a JPK 00901 instrument and Nano-Wizard software: Tapping mode, 10 nm tip radius, silicon tip, 292 KHz resonant frequency, 0.7-1 Hz scan speed, 256 x 256 and 512 x 512–pixels.

Transmission Electron Microscopy (TEM). Solutions of the gelator at specified concentration were drop-cast on carbon-coated copper grids and then carefully air-dried. Samples were stained with 0.1% uranyl acetate solution and the TEM images were taken at an accelerating voltage of 200 kV using a TECNAI F30 instrument.

Dynamic Light Scattering (DLS). 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 a dust-free conditions. Average hydrodynamic diameters (D_h) reported were obtained from the Gaussian analysis of the intensity-weighted particle size distributions.

Rheological Studies. For rheology experiment of the gels, an Anton Paar 100 rheometer with a cone and plate geometry (CP 25-2) having an adjustable peltier temperature controlling system was used. All the measurements were performed upon fixing the gap distance between the cone and the plate at 0.05 mm. The gels were scooped on the plate of the rheometer. An oscillatory strain amplitude sweep experiment was performed at a constant oscillation frequency of 1 Hz for the applied strain range 0.001-300 % at 20 °C. The software US-200 converted the torque measurements into either G' (the storage modulus) and G'' (the loss modulus) and represent G' and G'' with either strain or shear stress. Oscillatory frequency sweep experiments were performed in the linear viscoelastic region (strain 0.01%) to ensure that calculated parameters correspond to an intact network structures.

Polarized Optical Microscopy (POM). The changes in the birefringent textures of PyHis:PA = 1:1 hydrogel during gradual drying were followed under a polarized optical microscope (Olympus BX51) equipped with a heating stage (Mettler FP82HT) and a central processor (Mettler FP90).

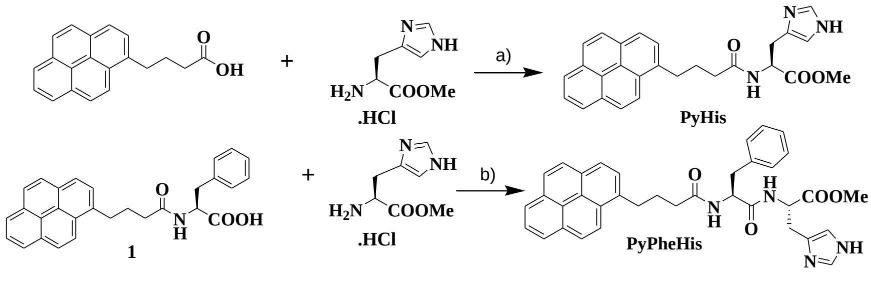
X-ray Diffraction. Gels were scooped onto glass slide and dried under vacuum for the corresponding XRD measurement. These samples were analyzed using a Bruker D8 Advance instrument (θ, 2θ geometry with Scintillation Detectors). The X-ray beam generated with a rotating Cu anode at the wavelength of KR beam at 1.5418Å was directed toward the film edge and scanning was done up to a 2θ value of 30°. Data were analyzed and interpreted using the Bragg equation.

Preparation of Shape-Persistent Gel Blocks. For this purpose, the needle attachment side of a glass barrel of a syringe (5 mL capacity) was cut carefully to make the barrel open from both sides. Then the hot sol (1 mL; [PyHis] = 23 mM) of the hydrogel has been poured into a barrel, which was prefixed with a plunger. The sol transformed into a stable gel when allowed to cool under ambient conditions for 15-20 min. The resultant 3-D gel block was then pushed carefully by pressing the plunger gradually, which eventually results in the construction of the gel blocks as shown in the manuscript.

Preparation of the Colored Gel Blocks. For this purpose, a solution of the water soluble dye, methylene blue (MB) has been prepared. The preformed hydrogel in the glass vial was heated to around 90 °C to make the sol/solution. MB solution was then added to the resultant hot sol. The blue colored sol thus obtained was poured into the barrel, which was prefixed with a plunger. The sol transformed into a stable colored gel when cooled under ambient condition for 1-2 min. The resultant blue colored gel block was then pushed carefully by pressing the plunger gradually, which eventually resulted in the formation of the colored gel blocks as shown in the manuscript.

2. Synthesis: The synthesis of compound **1** was carried out according to a reported procedure.[2] A synthetic scheme of PyHis and PyPheHis were shown below.

2a. General Synthetic Scheme:



Reagents, reaction conditions, and yields: a) DCC, HOBT, dry DCM, rt, 24 h, yield = 90%. b) DCC, HOBT, dry THF, rt, 24 h, yield = 65%.

2b. Materials and Methods: All chemicals, solvents and silica gel for TLC were obtained from well-known commercial sources and were used without further purification, as appropriate. Solvents were distilled and dried by standard procedure before use. Melting points was measured in open capillaries and were uncorrected. ^{1}H-NMR and ^{13}C-NMR spectra were recorded in Bruker-400 Advance NMR spectrometer. Chemical shifts were reported in ppm downfield from the internal standard tetramethylsilane (TMS). Mass spectrometry of individual compounds was performed using a MicroMass ESI-TOF MS instrument. Elemental analysis was recorded in Thermo Finnigan EA FLASH 1112 SERIES.

2c. Synthesis and Characterization:

Synthesis of PyHis: To a mixture of pyrene 1-butyric acid (1.5 g, 5.2 mmol), methyl ester of L-histidine hydrochloride (1.51 g, 6.24 mmol), DCC (1.62 g, 7.85 mmol) and HOBt (1.2 g, 7.84 mmol) in DCM (35 mL), Et_3N (3.6 mL, 26 mmol) was added at 0 °C under inert atmosphere and the mixtures were stirred at room temperature for 24 h. After completion of the reaction DCM was evaporated and the residual reaction mixtures were taken in ethyl acetate (50 mL). This resulted in the suspension of solid DCU (dicyclohexylurea), which was filtered off. The filtrate was dried and the purification of the residue was done using a silica gel column chromatography with 3% $MeOH/CHCl_3$ as the eluent. The product was obtained as an off white solid in 90% isolated yield. m.p. 195 °C; IR (Neat, cm^{-1}) 3404, 2977, 2944, 2604, 1740, 1626, 1476, 1172, 1050; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.21 (m, 2H), 2.39 (m, 2H), 3.07 (m, 2H), 3.38 (m, 2H), 3.72 (s, 3H), 4.86 (m, 1H), 6.79 (s, 1H), 7.5 (m, 1H), 7.87 (d, J = 8 Hz, 1H), 7.98 (m, 7H), 8.3 (d, J = 9.2 Hz, 1H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 27.32, 27.97, 32.52, 35.62, 52.09, 52.57, 123.4, 124.49, 124.56, 124.63, 124.68, 125.63, 126.33, 127.03, 127.23, 127.29, 128.4, 129.51, 130.63, 131.1, 134.29, 136.16, 171.69, 173.02; HRMS: *m/z* calcd. for $C_{27}H_{25}N_3O_3$ $(M+Na)^+$: 462.19, found: 462.1794; Elem. Anal.: calcd. for $C_{27}H_{25}N_3O_3 .0.5 H_2O$: C, 72.3; H, 5.84; N, 9.37; found: C, 72.61; H, 5.53; N, 9.21.

Synthesis of PyPheHis: To a mixture of compound **1** (0.7 g, 1.6 mmol), methyl ester of L-histidine hydrochloride (0.47 g, 1.9 mmol), DCC (0.5 g, 2.43 mmol) and HOBt (0.38 g, 2.45 mmol) in dry THF (30 mL), Et_3N (0.82 mL, 6.43 mmol) was added at 0 °C under inert atmosphere and the mixtures were stirred together at room temperature for 24 h. After the completion of the reaction as indicated by TLC, DCM was removed under vacuum and the reaction mixtures were taken up in ethyl acetate (50 mL) and the insoluble DCU (dicyclohexylurea) formed in the reaction was filtered off. The filtrate was dried and purification was done by silica column chromatography using 3-4% $MeOH/CHCl_3$ as the eluent. The product was obtained as an off-white solid in 65% yield. m.p. 165 °C; IR (Neat, cm^{-1}) 3277, 2926, 1742, 1648, 1535, 1438, 1212, 1180, 1020; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.13 (m, 2H), 2.26 (m, 2H), 2.97 (m, 4H), 3.3 (m, 2H), 3.65 (s, 3H), 4.72 (m, 1H), 4.74 (m, 1H), 6.71 (s, 1H), 7.16 (m, 5H), 7.43 (s, 1H), 7.79 (d, J = 7.6 Hz, 1H), 7.97 (m, 8H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 27.15, 28.3, 28.57, 32.5, 35.71, 52.32, 52.74, 54.3, 54.45, 123.17, 124.66, 124.8, 124.88, 125.74, 126.57, 126.86, 127.15, 127.21, 127.34, 128.42, 128.47, 129.16, 129.22, 129.75, 130.71, 131.24, 135.63, 136.44, 170.91, 171.57, 173.31; HRMS: *m/z* calcd. for $C_{36}H_{34}N_4O_4$ $(M+H)^+$: 587.26, found: 587.2658; Elemental analysis: calcd. for $C_{36}H_{34}N_4O_4$: C, 73.7; H, 5.84; N, 9.55; found: C, 73.53; H, 5.67; N, 9.71.

2d. Reference: 2. J. Nanda, A. Biswas, A. Banerjee, *Soft Matter* 2013, **9**, 4198.

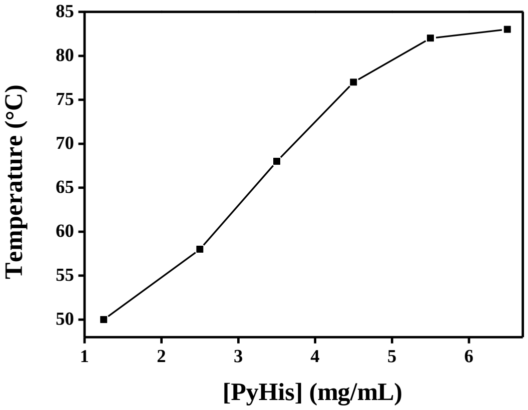


Figure S1. Concentration dependence of PyHis:PA = 1:1 gel melting temperature (T_{gel}).

Table S1. Summary of the pK_a values of different carboxylic acids and their salt formation and gel formation ability with PyHis in water.

Carboxylic Acids	pK_{a1}	pK_{a2}	**Salt Formation**	**Gel Formation**
Phthalic Acid (PA)	**2.98**[a]	5.28[a]	Yes	Yes
Isophthalic Acid (IPA)	3.46[a]	4.46[a]	No	No
Terephthalic Acid (TPA)	3.51[a]	4.82[a]	No	No
Homophthalic Acid	3.5[b]	4.3[b]	Yes	No
5-Nitro isophthalic Acid	**2.81**[c]		No	No
Benzoic Acid	4.17[a]	-	No	No
Acetic Acid	4.76[a]	-	No	No
Trifluoroacetic Acid	**-0.26**[a]	-	Yes	No
L-Tartaric Acid	**2.89**[a]	4.16[a]	Yes	No

(a)W. P. Jencks and J. Regenstein (1976) Ionization constants of acids and bases. In Handbook of Biochemistry and Molecular Biology. (Fasman, G.D., ed.), pp. 305-351. (b)W. L. F. Armarego, Christina Li Lin Chai, Purification of Laboratory Chemicals, Butterworth-Heinemann, 2003 - SCIENCE - 609 pages. (c)http://www.chemicalbook.com/ProductMSDSDetailCB7462421_EN.htm.

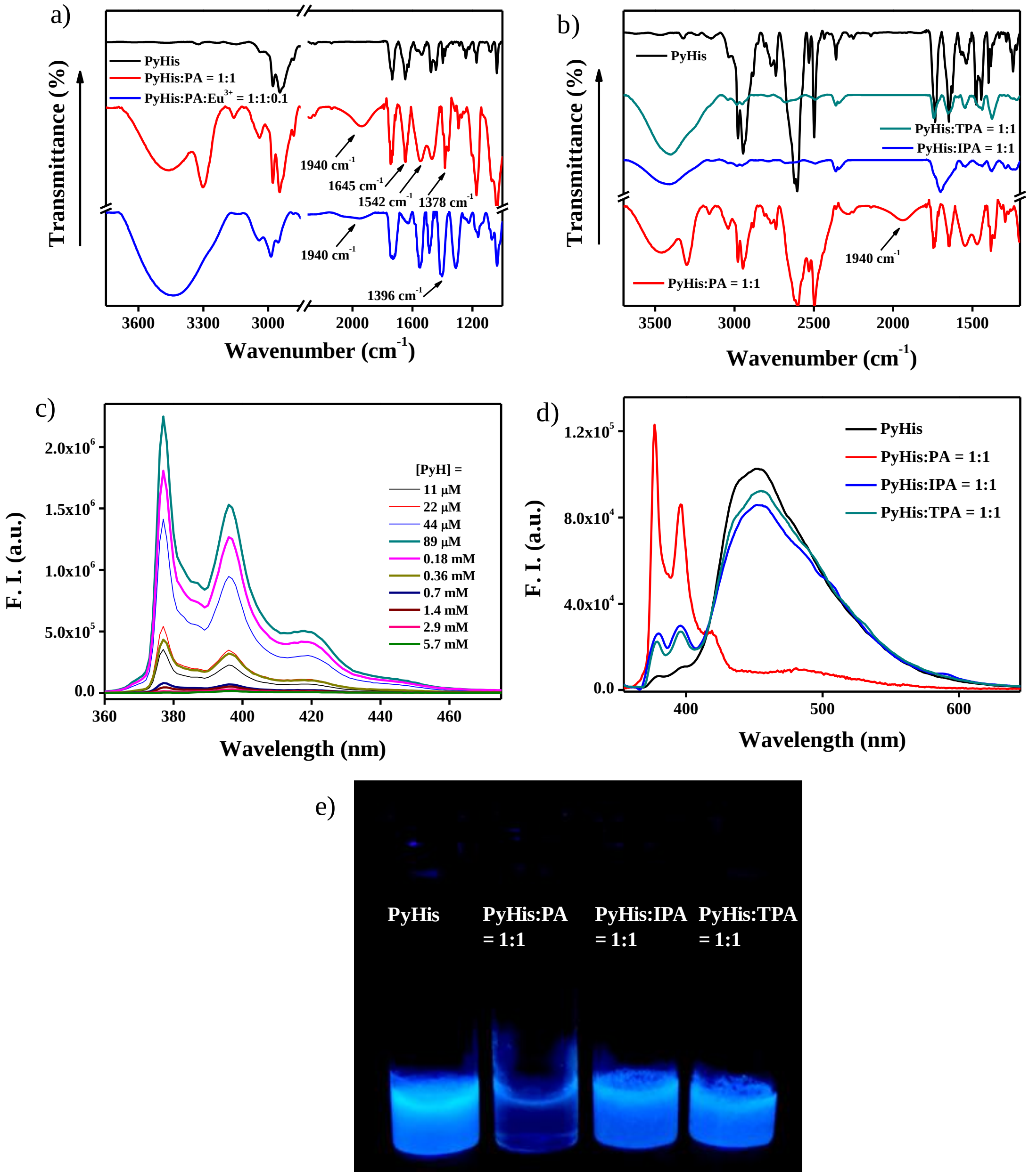


Figure S2. a) IR spectra of PyHis alone, PyHis:PA = 1:1 hydrogel and Eu^{3+} doped PyHis:PA = 1:1 hydrogel. b) Comparision of the IR spectra of PyHis:PA = 1:1 system with PyHis alone as well as PyHis:IPA = 1:1 and PyHis:TPA = 1:1; For this experiment the solution of each pair of the mixture or PyHis alone in water has been heated to 85-90 °C. Each suspension has been drop-casted on the CaF_2 cell and dried under vacuum before recording the spectra. c) Concentration dependent emission spectra of the PyHis:PA = 1:1 hydrogel. d,e) Emission spectra and the corresponding photographs of the solution of Py-His alone and its equimolar mixture with PA and IPA and TPA respectively from 9:1 H_2O/MeOH mixture.

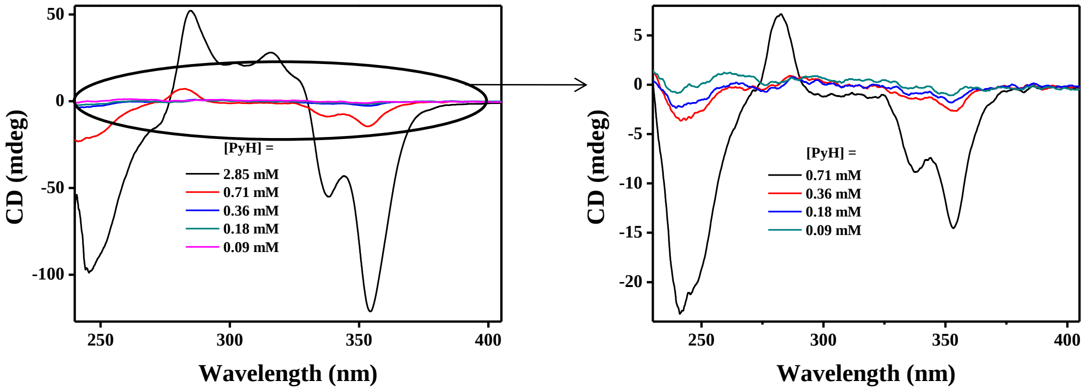


Figure S3. Concentration dependent CD spectra of the PyHis:PA = 1:1 hydrogel.

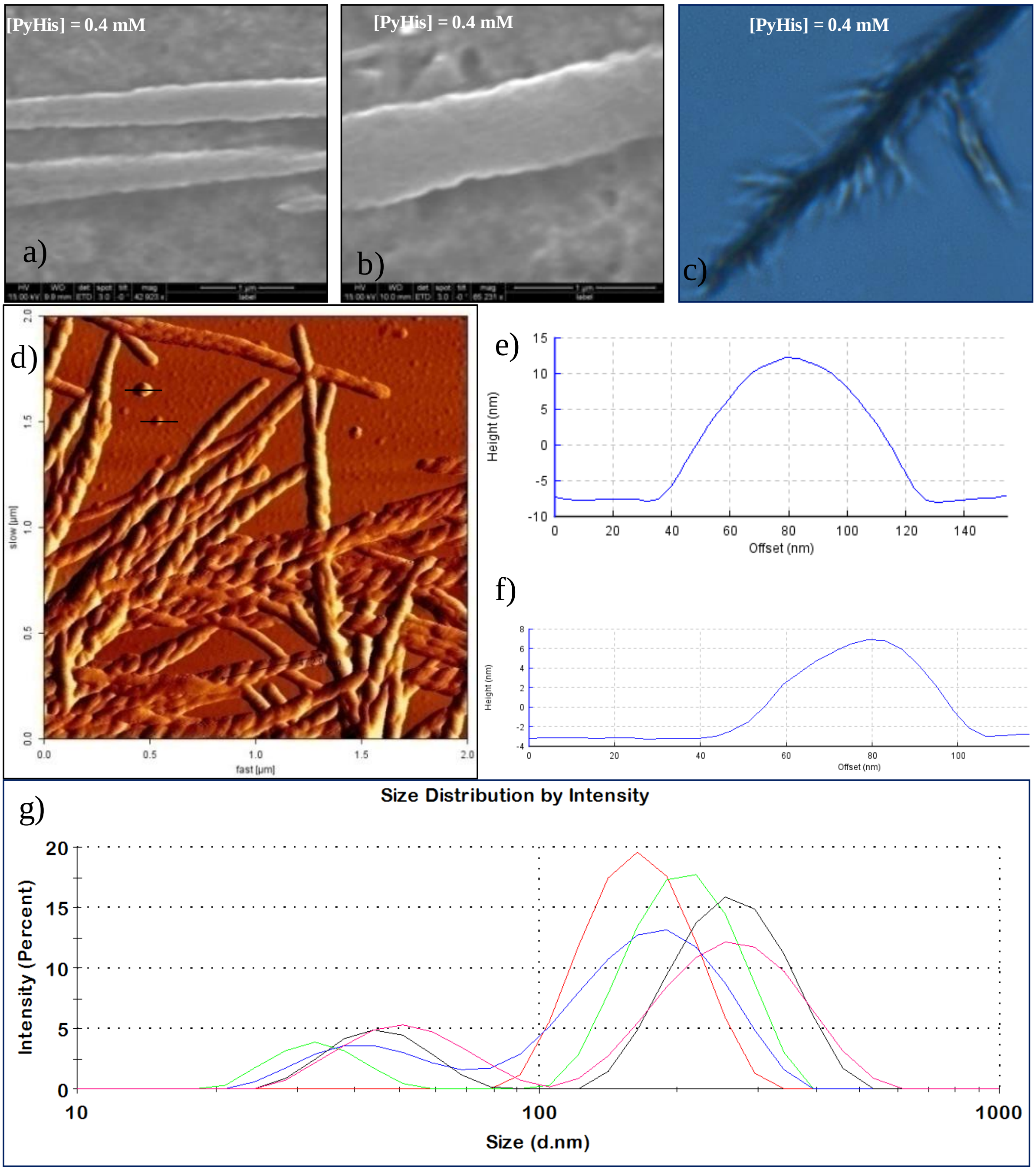


Figure S4. a,b) SEM images of the PyHis:PA = 1:1 system in water; [PyHis] = 0.4 mM. c) Fluorescence microscopic images of the PyHis:PA = 1:1 system, [PyHis] = 0.4 mM. d) Careful analysis of the AFM image showed the presence of multiple spherical aggregates along with the coiled-coil left-handed helical fibers and e,f) height images of two of such spherical aggregates were shown for clarity. g) DLS measurements of a solution of PyHis:PA = 1:1 system shows the average hydrodynamic diameter (D_h) of 276 ± 15 nm, [PyHis] = 0.2 mM.

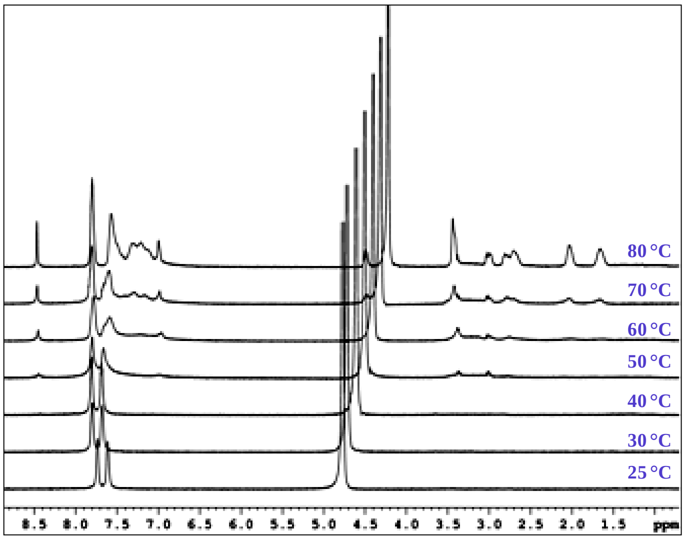


Figure S5. Variable temperature ^{1}H-NMR spectra of the PyHis:PA = 1:1 hydrogel; [PyHis] = 9.7 mM.

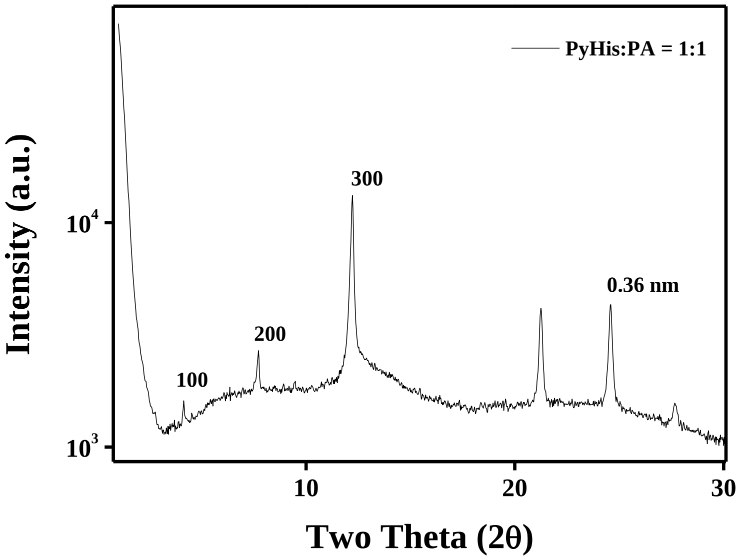


Figure S6. X-ray diffraction studies of the PyHis:PA = 1:1 hydrogel.

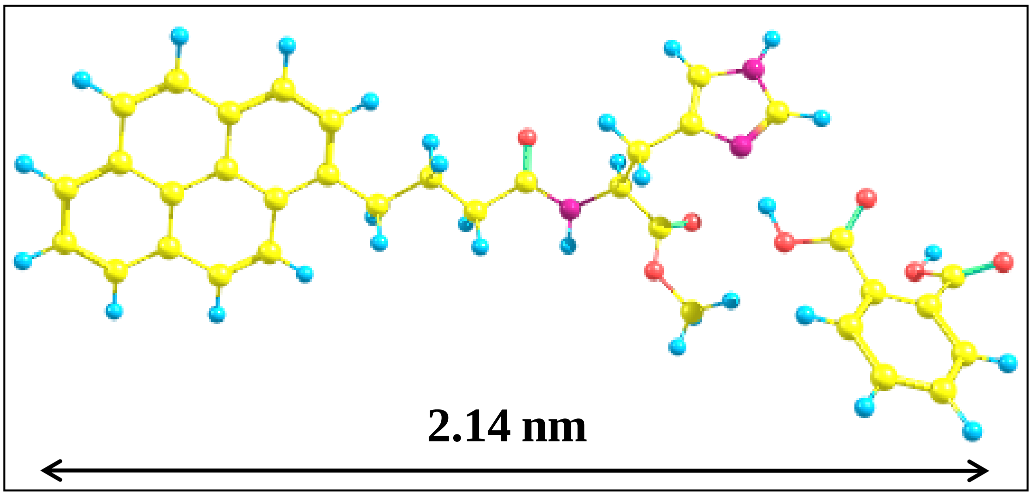


Figure S7. Energy minimized conformation of PyHis:PA = 1:1 salt as obtained using B3LYP/6-31G* level of computations.

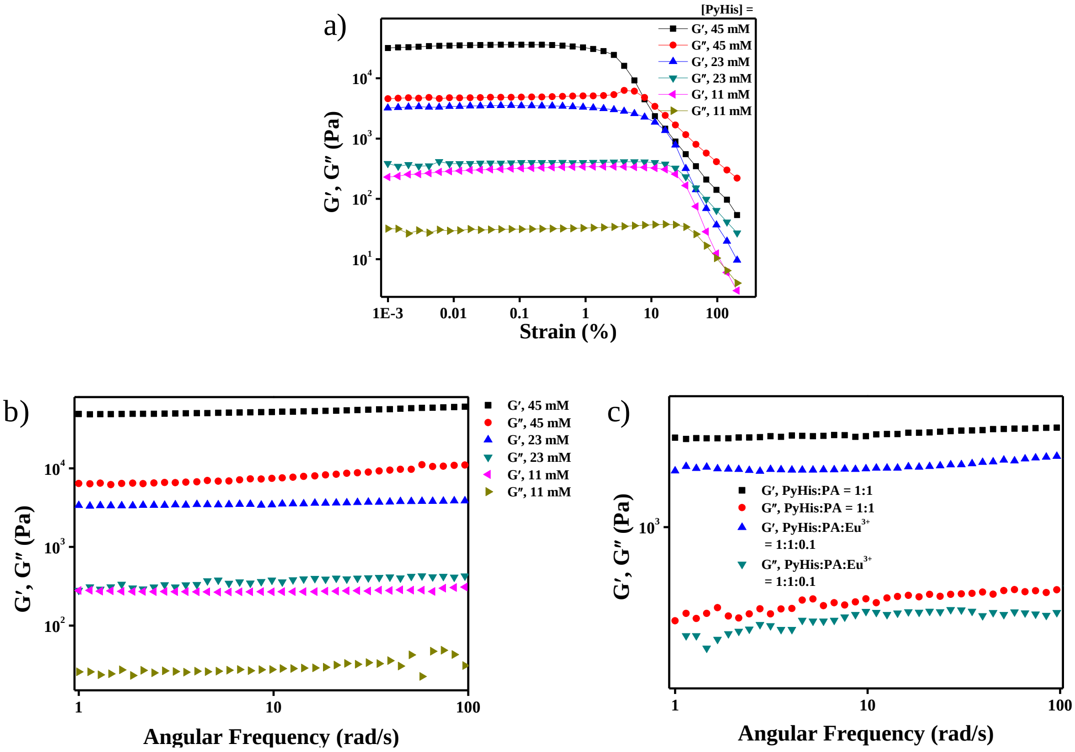


Figure S8. a,b) Oscillatory amplitude sweep and frequency sweep experiments of PyHis:PA = 1:1 hydrogel at three different concentrations respectively. c) Oscillatory frequency sweep experiments of the native gel and Eu^{3+} ion doped hydrogel; [PyHis] = [PA] = 23 mM.

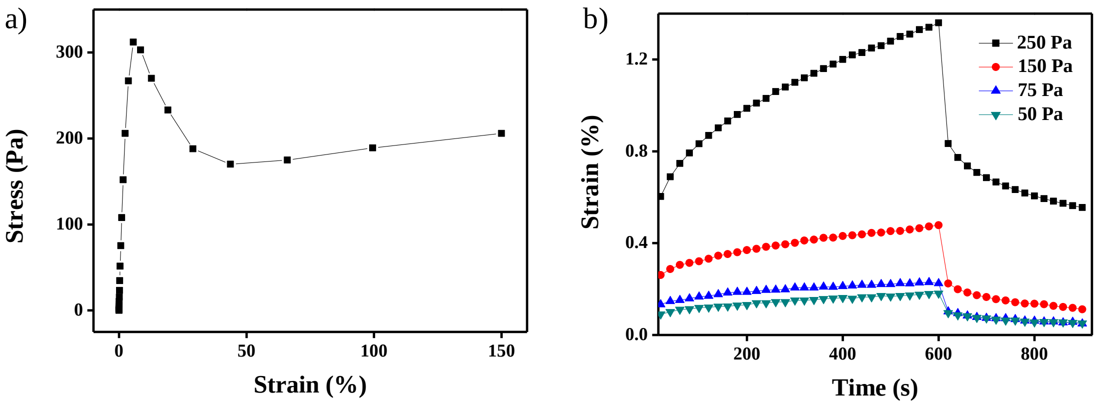


Figure S9. a) Stress-strain curve and b) Creep-recovery experiment at different stress levels of the PyHis:PA = 1:1 hydrogel; [PyHis] = [PA] = 30 mM.