Electronic Supplementary Material (ESI) for Soft Matter.

Electronic Supplementary Information

Aging effect on the enhancement of thermal stability, mechanical stiffness and fluorescence property of a histidine-appended naphthalenediimide-based two-component hydrogel

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Instrumentation

NMR experiments

All NMR studies were carried out on a Bruker DPX400 MHz or Bruker DPX500 MHz spectrometer at 300 K. Concentrations was in the range 5–10 mmol in DMSO-d_6.

Mass spectrometry

Mass spectra were recorded on a Q-Tofmicro^{TM} (Waters Corporation) mass spectrometer by a positive mode electrospray ionization process.

MALDI-TOF MS

MALDI-TOF MS analysis was performed using an Applied Biosystems MALDI-TOF Analyzer with dithranol as a matrix.

Fourier Transform Infrared (FTIR) study

All FT-IR spectra were recorded using the KBr pellet technique in a Nicolet 380 FT-IR spectrophotometer (Thermo Scientific).

Powder X-ray diffraction study (XRD) study

X-ray diffraction studies on the xerogels were carried out by placing the sample on a glass plate. Experiments were carried out using an X-ray diffractometer (Bruker AXS, Model D8 Advance). The instrument was operated at a 40 kV voltage and 40 mA current using Ni-filtered CuK_α radiation and the instrument was calibrated with a standard Al_2O_3 (corundum) sample before use. For scans over $2\theta = 1°-5°$, a scintillation counts detector was used with scan speed 2s and step size 0.02°. In another scan 2θ= 5°-50°, a Lynx Eye super speed detector was used with scan speed 0.3s and step size 0.02°.

Small-Angle X-ray Scattering (SAXS)

The measured SAXS intensity profile was fitted to a model of a core-shell cylinder using the software SASfit.[1]The fit parameters are the cylinder core radius R, with Gaussian polydispersity ΔR, shell thickness t, scattering contrast of coreη_c, scattering contrast of shellη_s, scattering contrast of solvent η_{solv}, and flat background BG (the length of the cylinder was fixed to $L = 100$ nm, this only affects the scaling of the scattering intensity since $L >> R$). The fitted values are $R =$ 4.11 nm, ΔR =1 nm (fixed), t = 10.33 nm, $\eta_c = 1.98 \times 10^{-2}$, $\eta_s = 3.99 \times 10^{-4}$, $\eta_{solv} = 1.14 \times 10^{-4}$ and BG= 7.04.

Rheology

The rheological experiments were carried out at 25ºC using a Anton Paar Modular Compact Rheometer (Model-MCR 102). Parallel plate 7 was used as measuring system.

Transmission electron microscopy (TEM)

TEM images were recorded on a JEM 2010 electron microscope at an accelerating voltage of

200 KV. 10 μL of tartaric acid based two component gel (concentration of NDIP is12.08 mM) or aggregates of NDIP alone/succinic acid based aggregates of NDIP was taken in a vial and diluted with 1ml milli-Q water. Then, a drop of dilute solution were placed on carbon coated copper grids (300 mesh) and dried by slow evaporation. Each grid was then allowed to dry in a vacuum for two days and then images were taken.

UV/Vis spectroscopy

UV/Vis absorption spectra were recorded on a Hewlett-Packard (model 8453) UV/Vis spectrophotometer (Varian Carry 50.bio) at 0.05mM concentration by dilutes the corresponding gel or aggregates.

PL spectroscopy

Fluorescence studies of the gel were carried out in a Perkin Elmer LS55Fluorescence Spectrometer instrument using the front face geometry. The sample was excited at 340 nm wavelength and emission scans were recorded from 350 to 750 nm. The gels or aggregates of NDIP with tartaric acid and succinic acid/*p*-nitrophenol were diluted to 0.5mM concentration with respect to NDIP and fluorescence experiments were carried out by taking those dilute solutions.

Time-Correlated Single Photon Counting (TCSPC)

TCSPC measurements were performed by Horiba JobinYvon IBH instrument having MCP

PMT Hamamatsu R3809 detector. TCSPC experiments were carried out by taking 2mM concentration of NDIP containing tartaric acid at various time intervals.

Experimental procedure

Synthesis procedure:

The synthesis of **NDIP** has been described previously by our research group. (Gayen, K.; Basu, K.; Nandi, N.; Das, K.S.; Hermida-Merino,D.; Hamley, I. W.;Banerjee,A. A Self-Assembled Peptide-Appended Naphthalene Diimide: A Fluorescent Switch for Sensing Acid and Base Vapors. *ChemPlusChem* **2019**, *84*, 1673–1680. (DOI: 10.1002/cplu.201900577)

Synthesis of Boc-11-aminoundecanoic acid (Boc-AUDA-COOH):

2.01g (10 mmol) of 11-aminoundecanoic acid (AUDA) was taken in a 250 ml round bottom flask. 10 ml 1(N) NaOH and 20 ml dioxane was added to it and cooled to 0°C. 2.20 g (10.1mmol) di-tert-butyl dicarbonate (Boc anhydride) was added to the reaction mixture and stirred for 8 hours at room temperature. Then dioxane was removed by reduced pressure. The resulting mixture was acidified with saturated $KHSO_4$ solution and the aqueous layer was extracted with ethyl acetate (3 x 40 mL). The ethyl acetate extract was dried over anhydrous sodium sulfate and evaporated in vacuum to obtain the colorless sticky product.

Yield: 2.71g (9 mmol, 90%)

Synthesis of Boc-AUDA-His-OMe (**P1**):

2.71 g (9 mmol) of Boc-AUDA-OH was dissolved in 15ml dry N,N-dimethyl formamide (DMF) and cooled in an ice bath. H-His-OMe (15mmol) was obtained by neutralization with saturated Na_2CO_3 from its hydrochloride salt and subsequent extraction with ethyl acetate. The ethyl acetate solution was then concentrated to 20 ml and added to the DMF solution followed by 1.35 g (10 mmol) of HOBt and 2.06 g (10 mmol) of N,N dicylohexylcarbodiimide (DCC).The reaction mixture was allowed to come at room temperature and stirred for 24 hr. The reaction mixture was diluted with ethyl acetate and filtered to separate N,N- dicyclohexyl urea (DCU). The ethyl acetate layer was washed with brine (2 × 30 ml). The organic layer was dried over anhydrous sodium sulfate and evaporated to obtain the yellowish product. The product was purified through silica gel column chromatography using methanol: chloroform (2:100) as eluent to obtain the pure white product. TLC was carried out with 3% methanol and chloroform.

Yield: 2.35 g (5.2 mmol, 57.7 %).

^{1}H NMR(500 MHz, $(CD_3)_2SO$, 25 °C): δ 8.91 (1H, s, amide-NH), 8.38-8.4 (1H, d, J = 8 Hz, amide-NH), 7.35 (1H, s, imidazole-CH of His), 6.72 (1H,brs, imidazole-CH of His), 4.59-4.57 (1H, m, α-CH of His), 3.62 (3H, s, $-OCH_3$ of ester), 3.16-2.86 (8H, m, 2 β-CH_2 of His, 4 α, α′-CH2, 2 β-CH_2 of AUDA), 1.43-1.39(3H, m,ω-CH_2 of AUDA), 1.36 (9H, s, 3 CH_3 of Boc), 1.21-1.16 (6 H, m,ω-CH_2 of AUDA). ^{13}C NMR (125 MHz, (CD3)2SO, 25 °C): δ 172.49, 171.23, 155.57, 133.73, 129.38, 116.94, 79.17, 77.27, 52.07, 51.17, 34.93, 29.46, 28.96, 28.84, 28.71, 28.69, 28.42, 28.25, 26.26, 26.12, 25.05. MALDI-TOF MS (m/z): Calculated, 452.30; observed 453.560 $[M+H]^+$, 475.538 $[M+Na]^+$, 491.531 $[M+K]^+$.

Synthesis of H2N-AUDA-His-OMe (**P2**):

To 2.26 g (5 mmol) of Boc-AUDA-His-OMe, 5 ml of 98% formic acid was added and the removal of the Boc group was monitored by TLC. After 6 h, formic acid was removed under vacuum. The residue was taken in water (8 ml) and pH of the aqueous solution was then adjusted to 8.0 with 30% aqueous NH_3. The aqueous portion was evaporated under vacuum. A white material was obtained, purified using basic alumina in and methanol chloroform (1:9) as eluent.

Yield: 1.58 g (4.5 mmol, 86.5 %).

^{1}H NMR (400 MHz, $(CD_3)_2SO$, 25 °C): δ 8.16-8.15(1H,d, J = 7.2 Hz, amide-NH), 7.50(1H,s, imidazole-CH of His), 6.76(1H, s,imidazole-CH of His), 4.47-4.41(1H, m, α-CH of His), 3.56 (3H, s, -OCH_3 of ester), 2.98-2.78 (4H, m, 2β-CH_2 of His, 2α$CH_2$of AUDA), 2.07-2.03 (2H, m,α′-$CH_2$of AUDA), 1.45-1.21 (16H, m, ω-CH_2 of AUDA). ^{13}C NMR (100 MHz, $(CD_3)_2SO$, 25 °C): δ 172.27, 172.23, 134.83, 52.30, 51.66, 41.55, 35.00, 33.11, 29.00, 28.95, 28.87, 28.74, 28.44, 26.40, 25.11.HRMS (m/z): Calculated, 352.25; observed 353.0811$[M+H]^+$.

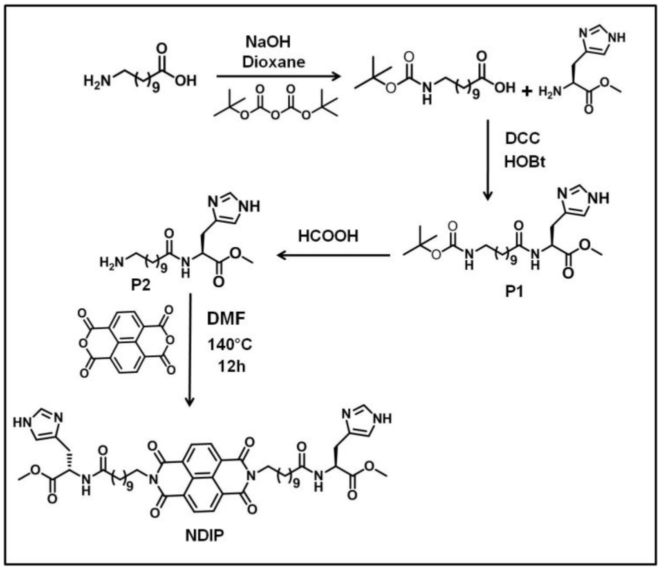
Synthesis of MeO-His-AUDA-NDI-AUDA-His-OMe (**NDIP**)

H_2N-AUDA-His-OMe (792 mg, 2.25 mmol) and 1,4,5,8-napthalenetetracarboxylicbisanhydride (268 mg, 1 mmol) were placed in a round-bottomed flask along with dry DMF (15 ml) and the reaction mixture was stirred for 12 h at 140 °C under N2 atmosphere. The heating was stopped and the solution was allowed to cool to room temperature and placed in the refrigerator for 30 min while the product came out as precipitate, which was filtered. The product was further purified by column chromatography using silica gel as stationary phase and methanol chloroform (1:9) as eluent. TLC was carried out with 5% methanol and 95% chloroform.

Yield: 768 mg (0.82 mmol, 82 %).

^1H NMR (500 MHz, $(CD_3)_2SO$, 25 °C): δ 11.81 (2H, brs, imidazole N-H), 8.63(4H, s, CH of NDI core), 8.12-8.10 (2H,d, J= 7.0 Hz, 2 amide-NH), 7.50 (2H, s, imidazole-CH of His), 6.77(2H, s, imidazole-CH of His),4.47-4.43(2H, m, α-CH of His), 4.04-4.01 (4H, m, 4α′$CH_2$of AUDA), 3.57 (6H, s, -OCH_3 of ester), 2.91-2.79 (8H, m, 4β-CH_2 of His, 4α-$CH_2$of AUDA), 2.07-2.04 (4H, m,4 β-CH_2 of AUDA), 1.66-1.63 (4H, m, ω-CH2 of AUDA), 1.45-1.23 (20H, m, ω-CH2 of AUDA). ^{13}C NMR (125 MHz, $(CD_3)_2SO$, 25 °C): δ 172.19, 172.13, 162.50, 134.74, 130.34, 126.18, 126.06, 52.22, 51.59, 34.97, 28.77, 28.65, 28.62, 28.38, 27.39, 26.43, 25.05. MALDI-TOF MS (m/z): Calculated, 936.47; observed 937.405$[M+H]^+$, 959.369$[M+Na]^+$, 975.430$[M+K]^+$.

Synthetic Scheme



Scheme 1: Synthetic scheme for the molecule **NDIP**.

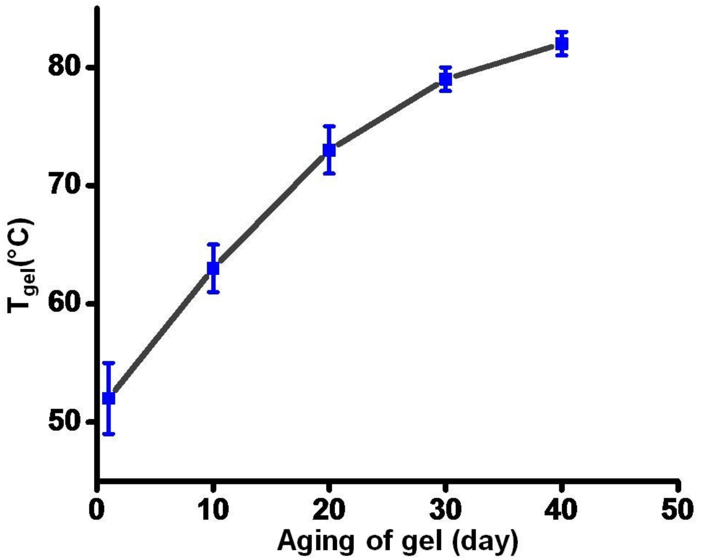


Fig. S1 Plot of T_{gel} vs aging of gel (fresh gel to 40 days aged gel) with the error bars.

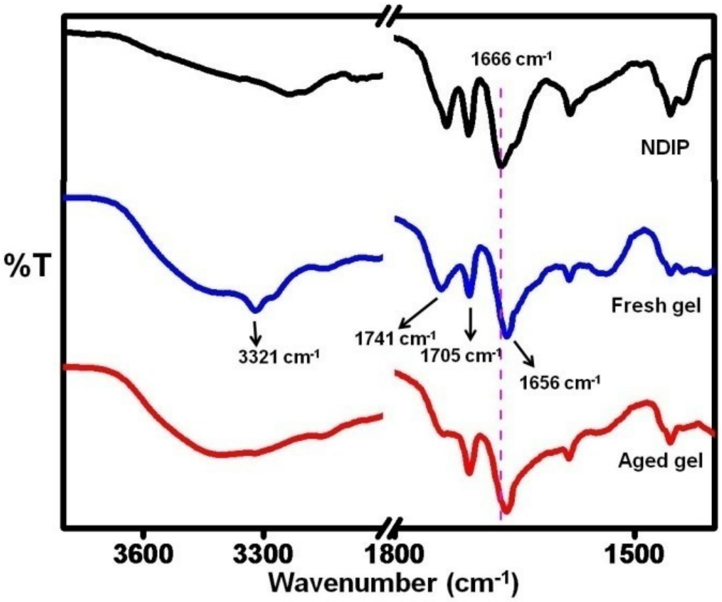


Fig. S2 FT-IR spectra of **NDIP**, tartaric acid and **NDIP** based two component hydrogel (freshly prepared and 40 days aged hydrogel)

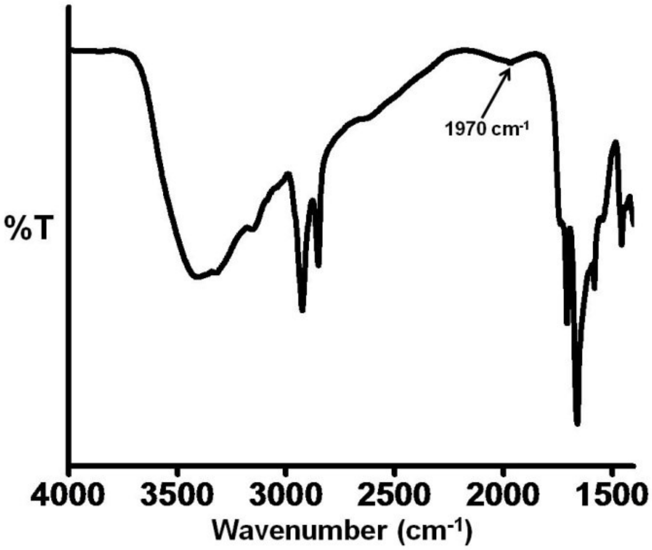


Fig. S3 FT-IR spectra of **NDIP** and tartaric acid based two component aged hydrogel (40 days). The peak indicates acid-base interaction of carboxylic acid group of tartaric acid and imidazole ring of **NDIP**.

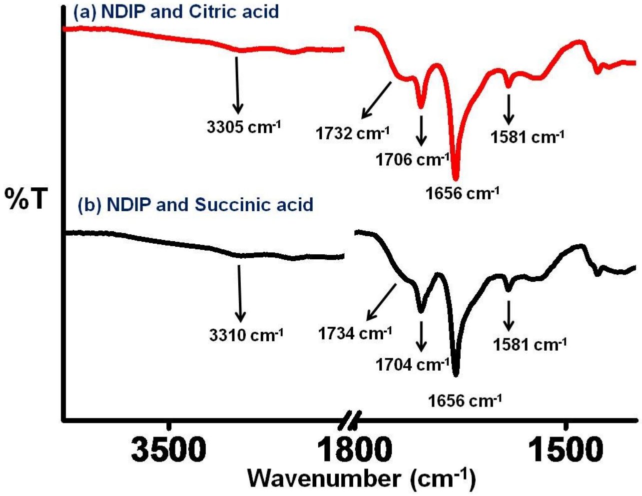


Fig. S4 FT-IR spectra of (a) **NDIP** and citric acid (b) **NDIP** and succinic acid based two component aggregates.

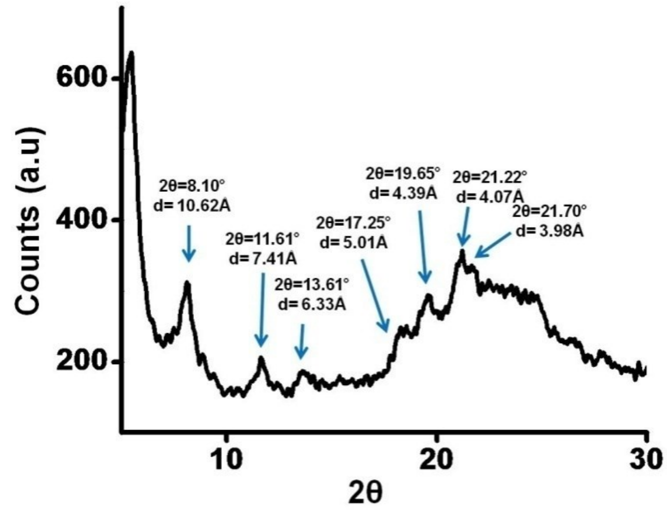


Fig. S5 Wide-angle powder X-ray diffraction (WPXRD) spectra of **NDIP** in the aggregated state in ultrapure water.

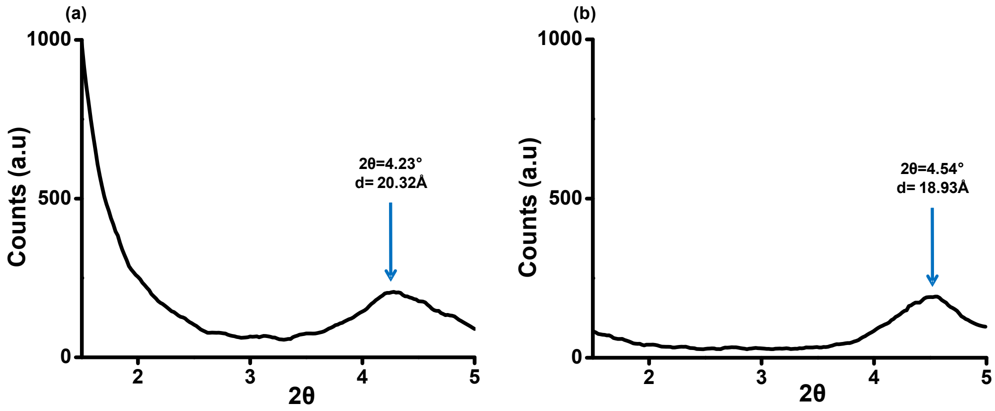


Fig. S6 Low-angle powder X-ray diffraction (WPXRD) spectra of **NDIP** and tartaric acid based two component (a) freshly prepared and (b) 40 days aged hydrogel.

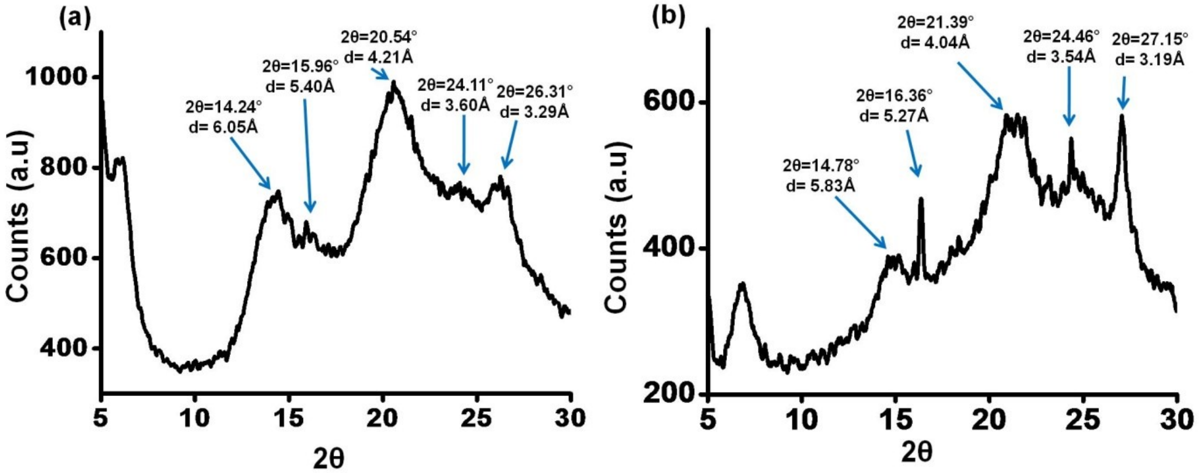


Fig. S7: Wide-angle powder X-ray diffraction (WPXRD) spectra of **NDIP** and tartaric acid based two component (a) freshly prepared and (b) 40 days aged hydrogel.

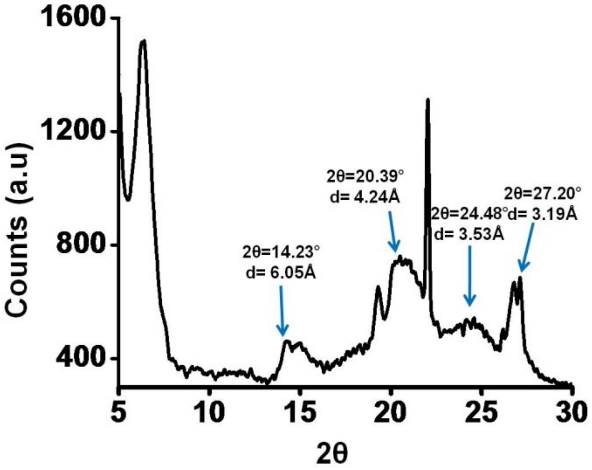


Fig. S8: Wide-angle powder X-ray diffraction (WPXRD) spectra of **NDIP** and succinic acid based two component aggregates

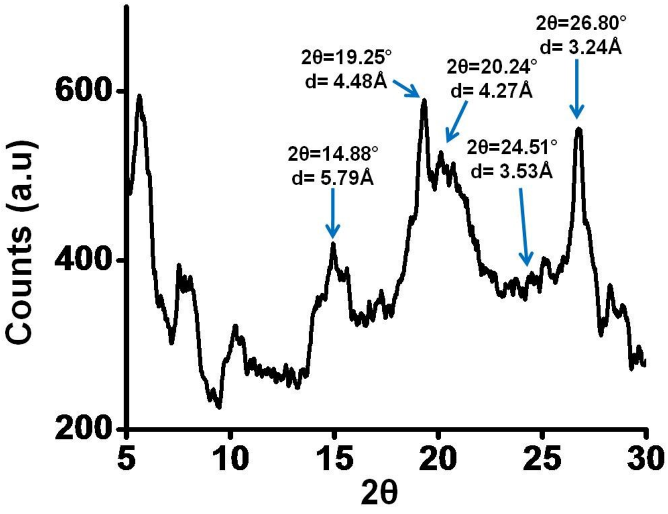


Fig. S9: Wide-angle powder X-ray diffraction (WPXRD) spectra of **NDIP** and citric acid based two component aggregates

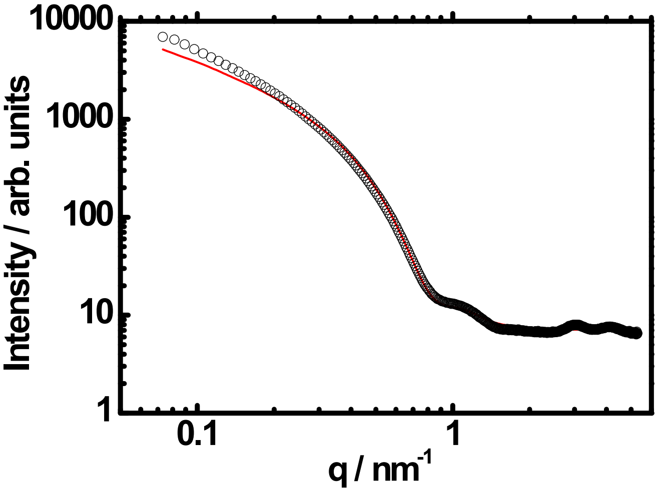


Fig. S10 Small angle X-ray Scattering (SAXS) data for **NDIP** and tartaric acid-based two-component hydrogel (after 20 days). The open circles are measured data (only every third point is shown for convenience) and the red line is a model form factor fit as described above.

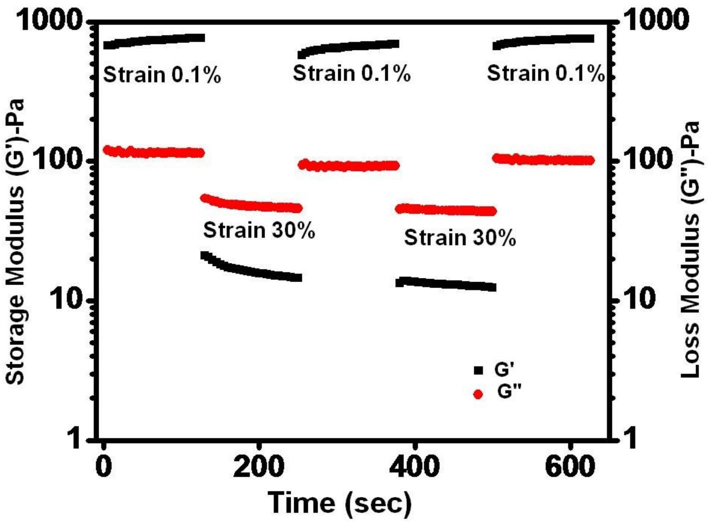


Fig. S11 Strain sweep rheology data of **NDIP** and tartaric acid based two component hydrogel after 20 days.

Fig. S12 Thixotropic behavior of **NDIP** and tartaric acid-based two-component hydrogel

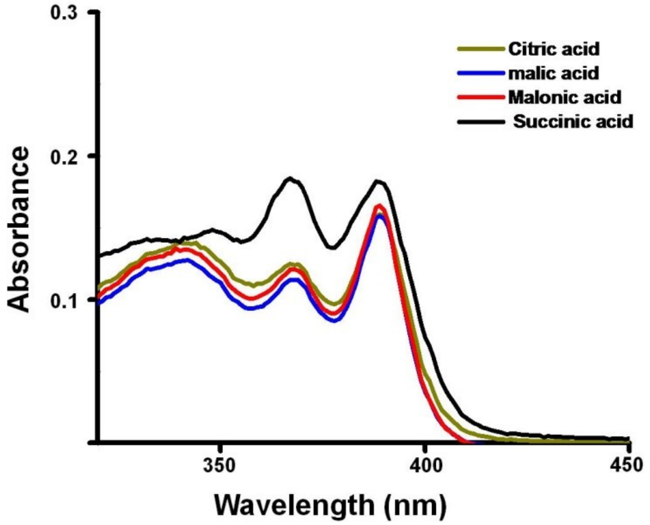


Fig. S13 UV-Vis spectra of **NDIP** in presence of different dicarboxylic acid (citric acid, malic acid, malonic acid, succinic acid).

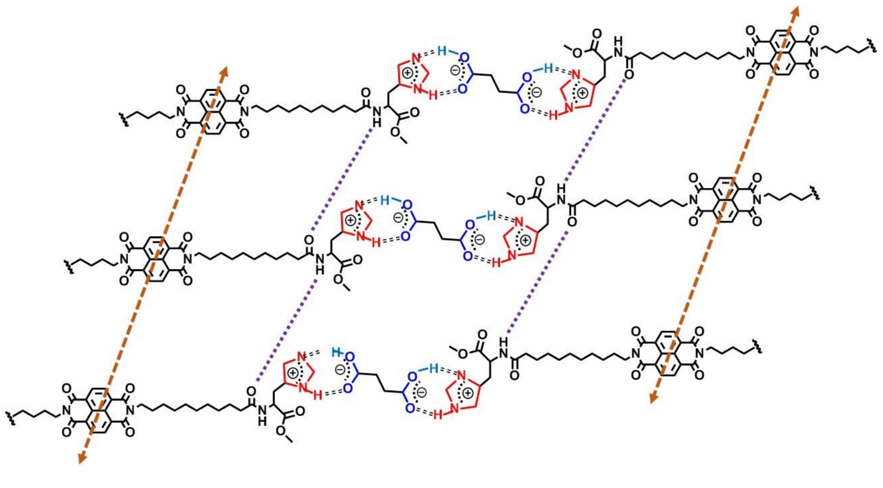


Fig. S14 Probable packing model for NDIP and succinic acid based two component self-assembled system in aqueous medium

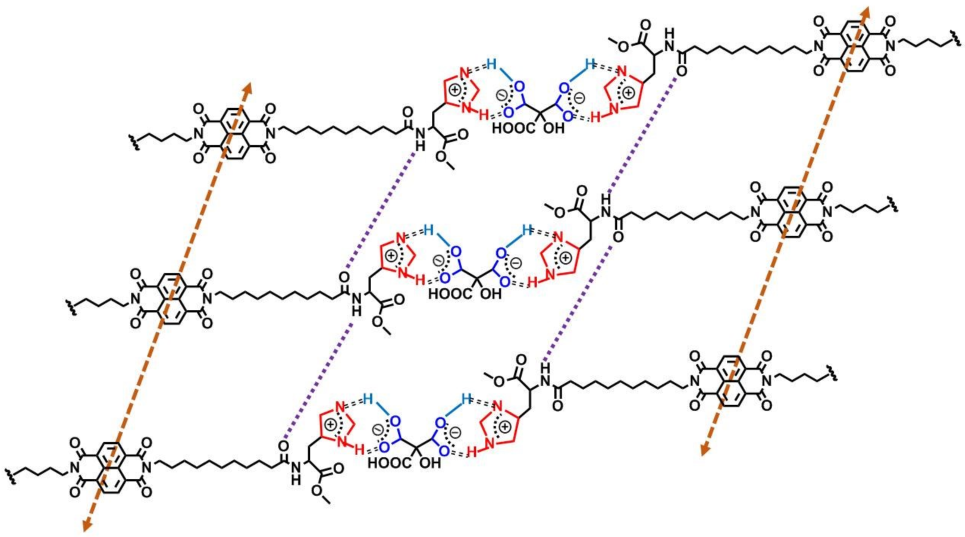


Fig. S15 Probable packing model for NDIP and citric acid based two component self-assembled system in aqueous medium

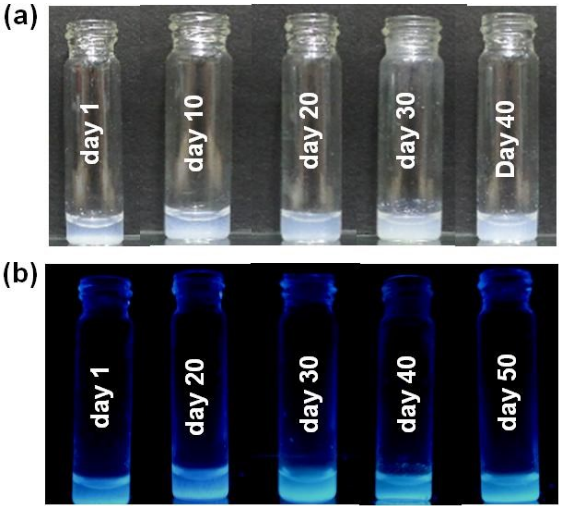


Fig. S16 (a) The visual appearance and (b) fluorescence image of aggregates of **NDIP** in ultrapure water.

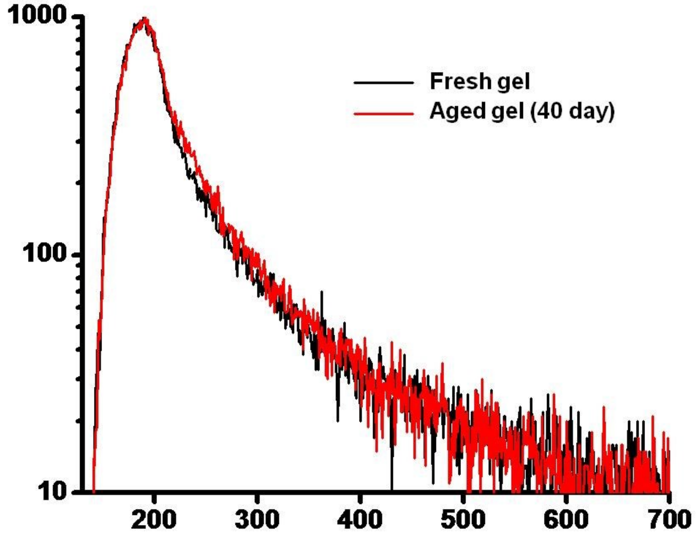


Fig. S17 Time-correlated Single Photon Counting (TCSPC) spectra of **NDIP** and tartaric acid based two component gel (freshgel and 40 days aged gel)

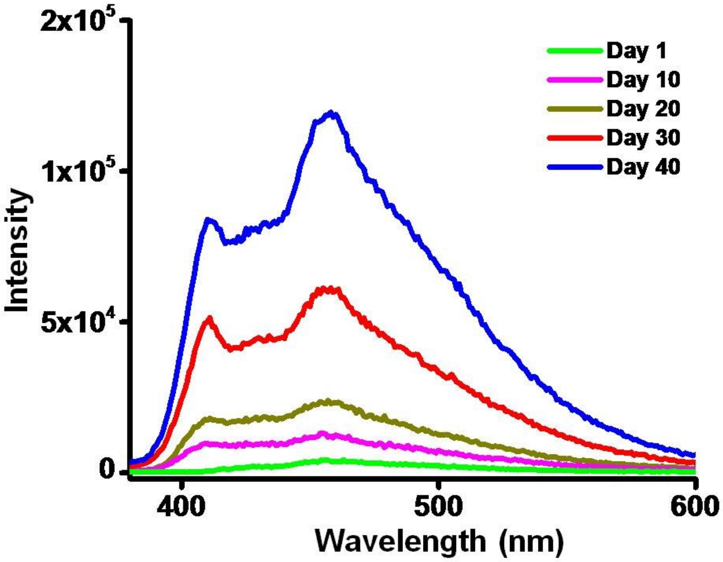


Fig. 18 Time dependent fluorescence spectra of NDIP and tartaric acid at a molar ratio 1:0.5

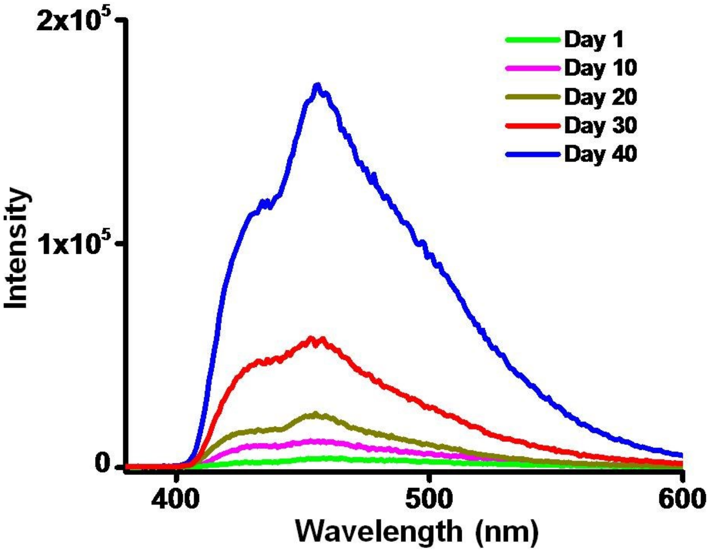


Fig. 19 Time dependent fluorescence spectra of NDIP and tartaric acid at a molar ratio 1:1.

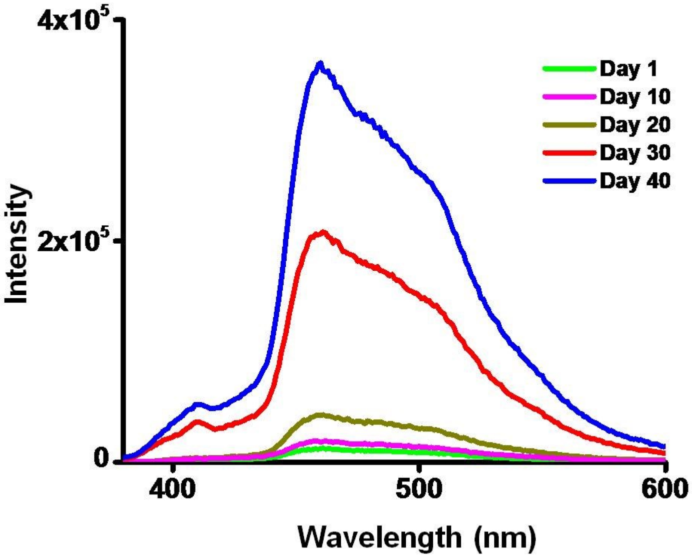


Fig. 20 Time dependent fluorescence spectra of NDIP and tartaric acid at a molar ratio 1:4.

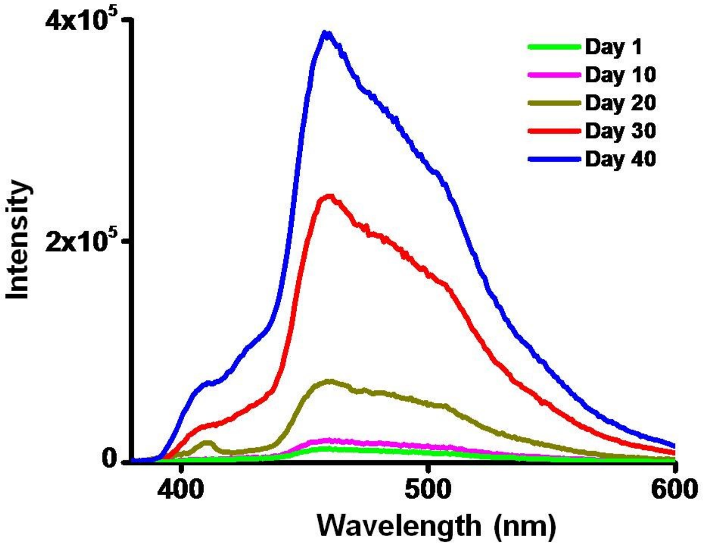


Fig. 21 Time dependent fluorescence spectra of NDIP and tartaric acid at a molar ratio 1:8.

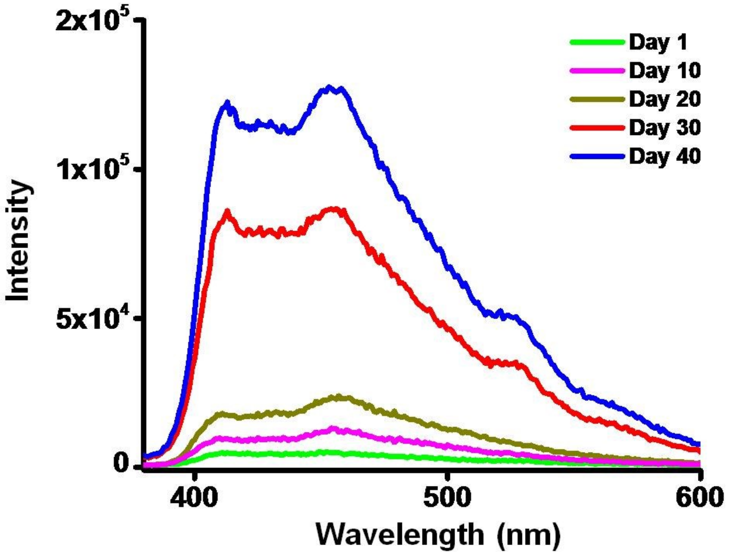


Fig. 22 Time dependent fluorescence spectra of NDIP and *p*-nitrophenol (PNP) at a molar ratio 1:2.

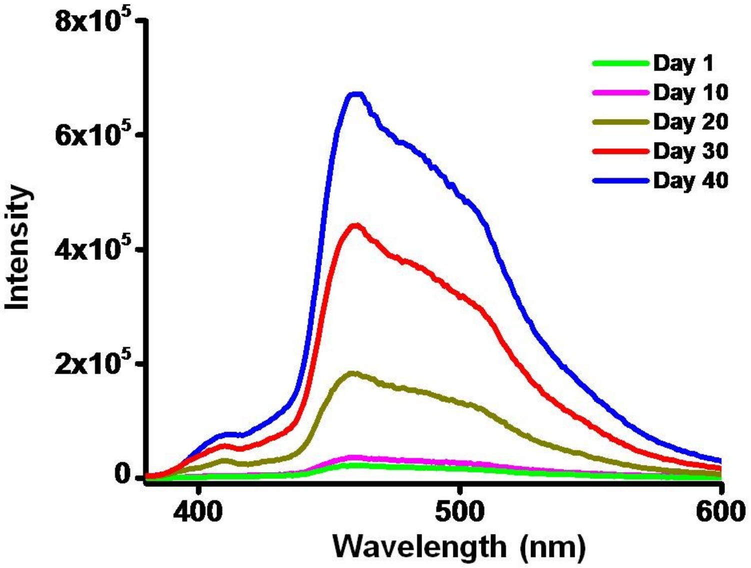


Fig. 23 Time dependent fluorescence spectra of NDIP and oxalic acid at a molar ratio 1:2.

1. Bressler, I.; Kohlbrecher, J.; Thünemann, A. F., *J. Appl. Crystallogr.* **2015,** *48*, 1587.