Electronic Supplementary Information

Time-Dependent Gel to Gel Transformation of a Peptide based Supramolecular Gelator

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Experimental Section

Synthesis of the gelator peptide

The dipeptide was synthesized by conventional solution phase methods by using racemization free fragment condensation strategy. Coupling was mediated by N,N-dicyclohexylcarbodiimide and 1-hydroxybenzotriazole monohydrate (DCC/HOBt.H_2O). C-terminal methyl group was deprotected by using aqueous sodium hydroxide. The final compound was fully characterized by mass spectrometry, ^{1}H NMR spectroscopy and ^{13}C NMR spectroscopy (Figure S1-S3).

Synthesis of Myr-Ala-OMe: 2.28 g (10 mmol) of Myr-COOH was dissolved in 12 mL dry N,N-dimethyl formamide (DMF) and cooled in an ice bath. H-Ala-OMe 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 10 mL and added to the DMF solution followed by 1.53 g (10 mmol) of HOBt.H_2O and 2.06 g (10 mmol) of N,N-dicylohexylcarbodiimide (DCC). The reaction mixture was allowed to come at room temperature and it was stirred for 24 hours. The reaction mixture was diluted with ethyl acetate and filtered to separate N,N- dicyclohexyl urea (DCU). The ethyl acetate layer was washed with 1(N) HCl (3 x 30 mL), brine (2 x 30 mL), saturated sodium carbonate solution (2 x 30 mL) and brine (2 x 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 petroleum ether (60 °C-80 °C)/ethyl acetate (5:1) as eluent to obtain the pure white product. Yield: 2.02 g (6.45 mmol, 64.5 %,).

^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.06-6.05 (1H, d, NH, J = 6.5 Hz), 4.62-4.58 (1H, m, α-H of Ala), 3.74 (3H, s, $-OCH_3$), 2.20-2.18 (2H, t, α-CH_2 of Myr, J = 7.5 Hz), 1.64-1.58 (2H, m, β-CH_2 of Myr), 1.39-1.38 (3H, d, β-Hs of Ala, J = 7.5 Hz), 1.28-1.20 (20H, m, chain CH_2-s of Myr), 0.88-0.85 (3H, t, $-CH_3$ of Myr, J = 7.0 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.82, 172.82, 52.50, 52.46, 47.93, 36.60, 31.99, 29.72, 29.70, 29.56, 29.43, 29.31, 25.67, 22.76, 18.59, 14.18. HRMS (m/z): Calculated for $C_{18}H_{35}NO_3$: 313.2617, Found: 314.1449 $(M+H)^+$,336.1183 $(M+Na)^+$, 352.0808 $(M+K)^+$.

Synthesis of Myr-Ala-OH: 2.0 g (6.38 mmol) of Myr-Ala-OMe was taken in a round bottom flask and it was dissolved in 50 mL methanol. 12 mL of 1 (N) NaOH was added to it and kept under stirring for 6 hours. The progress of hydrolysis was monitored by thin layer chromatography (TLC). After the completion of the reaction, as indicated by TLC, the methanol was removed under vacuum. The aqueous part was then taken in 50 mL water and washed with diethyl ether (2 x 30 mL). The remaining solution was acidified with 1 (N) HCl and extracted with with ethyl acetate (3 x 40 mL). The ethyl acetate extract was dried over anhydrous sodium sulfate and evaporated in vacuum to obtain a white powdered product. Yield: 1.81 g (6.05 mmol, 94.83 %).

^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.40 (1H, brs, −COOH), 8.05-8.03 (1H, d, NH, J= 7.2 Hz), 4.20-4.15 (1H, m, α-H of Ala), 2.10-2.05 (2H, t, α-CH_2 of Myr, J= 7.4 Hz), 1.48-1.46 (2H, m, β-CH_2 of Myr), 1.23 (26H, m, chain CH_2-s of Myr and β-Hs of Ala), 0.87-0.83 (3H, t, −CH_3 of Myr, J= 6.4 Hz). ^{13}C NMR (75 MHz, DMSO- d_6) δ 174.84, 172.51, 47.84, 35.54, 31.88, 29.61, 29.55, 29.40, 29.30, 29.19, 25.74, 22.66, 17.74, 14.47. HRMS (m/z): Calculated for $C_{17}H_{33}NO_3$: 299.449, Found: 322.4741 $(M+Na)^+$, 338.4594 $(M+K)^+$.

Synthesis of Myr-Ala-Ala-OMe: 1.8 g (6.0 mmol) of Myr-Ala-OH was dissolved in 8 mL dry N,N-dimethyl formamide (DMF) and cooled in an ice bath. H-Ala-OMe 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 8 mL and added to the DMF solution followed by 0.92 g (6.03 mmol) of HOBt.H_2O and 1.29 g (6.3 mmol) of N,N-dicylohexylcarbodiimide (DCC). The reaction mixture was allowed to come at room temperature and stirred for 24 hours. The reaction mixture was diluted with ethyl acetate and it was then filtered to separate N,N- dicyclohexyl urea (DCU). The ethyl acetate layer was washed with 1(N) HCl (3 x 30 mL), brine (2 x 30 mL), saturated sodium carbonate solution (2 x 30 mL) and brine (2 x 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 petroleum ether (60 °C-80 °C)/ethyl acetate (4:1) as eluent to obtain the pure white product. Yield: 1.51 g (3.94 mmol, 65.67 %).

^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.87 (1H, brs, NH), 6.27 (1H, brs, NH), 4.54-4.52 (2H, m, α-H of Ala), 3.74 (3H, s, −OCH_3), 2.21-2.18 (2H, t, α-CH_2 of Myr, J= 7.5 Hz), 1.60 (2H, brs, β-CH_2 of Myr), 1.41-1.35 (6H, m, β-Hs of Ala), 1.27-1.24 (20H, m, chain CH_2-s of Myr), 0.88-0.86 (3H, t, −CH_3 of Myr, J= 7.0 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.39, 173.16, 172.30, 52.56, 48.69, 48.27, 36.68, 32.04, 29.76, 29.73, 29.60, 29.46, 29.35, 25.75, 22.79, 18.60, 18.08, 14.21. HRMS (m/z): Calculated for $C_{21}H_{40}N_2O_4$: 384.5533, Found: 407.6336 $(M+Na)^+$, 423.6201 $(M+K)^+$.

Synthesis of Myr-Ala-Ala-OH (MAA) : 1.32 g (3.43 mmol) of Myr-Ala-Ala-OMe was taken in a round bottomed flask and dissolved in 50 mL methanol. 8 mL of 1 (N) NaOH was added to it and kept under stirring for 6 hours. The progress of hydrolysis was monitored by thin layer chromatography (TLC). After the completion of the reaction, as indicated by TLC, the methanol was removed in vacuum. The aqueous part was then taken in 50 mL water and washed with diethyl ether (2 x 30 mL). The remaining solution was acidified with 1 (N) HCl and extracted with ethyl acetate (3 x 40 mL). The ethyl acetate extract was dried over anhydrous sodium sulfate and evaporated in vacuum to obtain a white powdered product. Yield: 1.22 g (3.29 mmol, 96.13 %).

^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.51 (1H, br, −COOH), 8.09-8.04 (1H, m, NH), 7.92-7.88 (1H, m, NH), 4.35-4.28 (1H, m, α-H of Ala), 4.23-4.15 (1H, m, α-H of Ala), 2.11-2.05 (2H, m, α-CH_2 of Myr), 1.45 (2H, brs, β-CH_2 of Myr), 1.27-1.16 (26H, m, chain CH_2-s of Myr and β-Hs of Ala), 0.87-0.82 (3H, t, −CH_3 of Myr, J= 6.5 Hz). ^{13}C NMR (75 MHz, DMSO- d_6) δ: 174.54, 174.48, 172.75, 172.43, 48.16, 47.92, 35.63, 31.86, 29.58, 29.36, 29.27, 29.19, 25.76, 22.65, 18.76, 17.68, 14.50. HRMS (m/z): Calculated for $C_{20}H_{38}N_2O_4$: 370.5267, Found: 371.0818 $(M+H)^+$, 393.0415 $(M+Na)^+$, 409.0248 $(M+K)^+$.

Instrumentation

Field emission scanning electron microscopic (FE-SEM) study: FE-SEM experiments were performed by placing a small portion of gel samples on a microscope cover glass. Then, these samples were dried first in air and then in vacuum and coated with platinum for 90 s at 10 kV voltages and 10 mA current.

The average thickness of the coating layer of platinum was 3 to 4 nm. After that micrographs were taken by using a Jeol Scanning Microscope JSM-6700F.

Transmission electron microscopy (TEM) study: The morphology of the hydrogel was investigated by using a transmission electron microscope. The samples were prepared by depositing dilute gel sample onto a TEM grid (300 mesh Cu grid). Then, the grid was first air-dried and then under vacuum at 30 °C for one days. Images were recorded on a JEOL electron microscope at an accelerating voltage of 200 kV.

Small and Wide Angle Powder X-ray diffraction study: X-ray diffraction study of the xerogel was carried out by placing the sample on a glass plate. Experiments were carried out by using an X-ray diffractometer (Bruker AXS, Model No. D8 Advance). The instrument was operated at a 40 kV voltages 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 scan 5°–30°, the LynxEye super speed detector was used with scan speed 0.5 s and step size 0.02°.

Small and Wide Angle X-Ray Scattering (SAXS): SAXS Measurements were performed using a Bruker Nanostar instrument using CuK_{α} radiation and a Vantec 2000 detector. The sample-to-detector distance was 1.07 m. The $q = 4\pi\sin\theta/\lambda$ (scattering angle 2θ) scale was calibrated using silver behenate. Samples were mounted in quartz capillaries. WAXS were carried out on a BM26B DUBBLE at the ESRF, Grenoble, France.

FTIR spectroscopy: The FTIR spectrum of the xerogel were recorded on a Shimadzu (Japan) FTIR spectrophotometer. In the solid-state FTIR studies, the powdered samples were mixed with KBr to prepare the thin films.

Fluorescence spectroscopy: The fluorescence spectra were recorded on a Perkin–Elmer LS55 Fluorescence Spectrometer. The pyrene containing gel samples were placed in a quartz cell (path length: 0.5 cm) and excited at 334 nm. The emission scans were recorded from 344–600 nm by using a slit width of 10 nm for excitation and 5 nm for emission slits.

UV/Vis spectroscopy: UV/Vis absorption spectra were recorded on a hewlett-packard (model 8453) UV/Vis spectrophotometer (varian carry 50.bio).

Rheology: The rheology experiment was performed by using an AR 2000 advanced rheometer (TA Instruments) using cone-plate geometry in a Peltier plate.

NMR experiments: All NMR studies were carried out on a Brüker DPX 300 MHz and Brüker DPX 500 MHz spectrometers at 300 K. Concentrations were in the range 1–10 mM in $CDCl_3$ or DMSO-d_6.

Mass spectrometry: Mass spectra were recorded on a Q-Tof microTM (Waters Corporation) mass spectrometer by positive mode electro spray ionization process.

Differential scanning calorimetry (DSC): Thermal behavior of the gel was studied by using a Perkin-Elmer differential scanning calorimeter (Diamond DSC-7) working under N_2 atmosphere. Gel sample was taken in large volume capsule (LVC) tightly sealed with an O-ring. Gel sample was then heated from 25 °C to 85 °C at the heating rate of 5 °C min^{-1}. A cooling run was also taken after waiting 10 min at 85 °C with a cooling rate of 5 °C min^{-1} to 20 °C. The instrument was calibrated with indium and cyclohexane.

Melting point: The melting point of the xerogels obtained for both the freshly prepared and the aged gels were measured by using a commercially available melting point apparatus and it has been found that both xerogels exhibited a melting point of 112 °C.

Figures

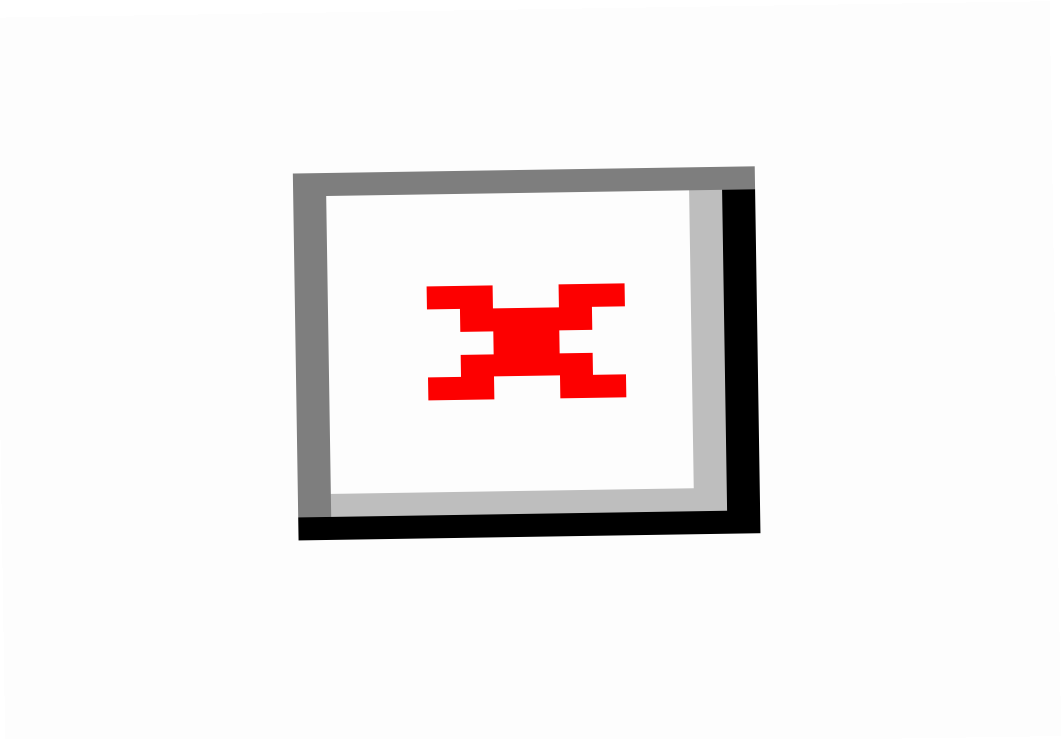

Fig. S1 300 MHz ^{1}H-NMR spectrum of the gelator peptide **MAA**.

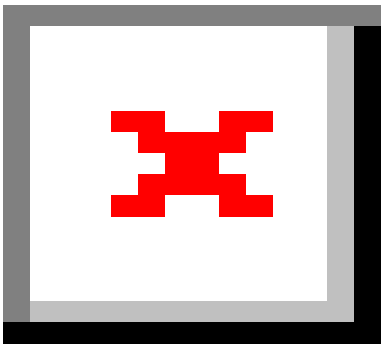

Fig. S2 75 MHz ^{13}C-NMR spectrum of the gelator peptide **MAA**.

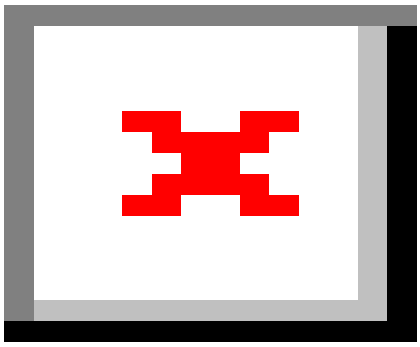

Fig. S3 Mass spectrum of the gelator peptide **MAA**.

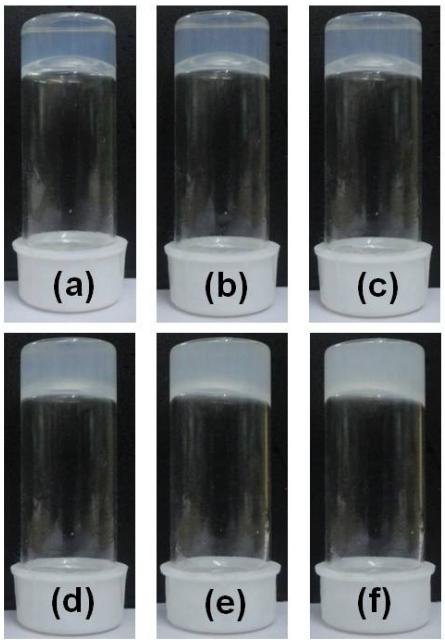

Fig. S4 Faster transformtion of one gel form (transparent) to another gel form (turbid) at higher concentration of 6.0 mg/mL (0.6 %(w/v)) of **MAA** at time intervals (a) 25 minutes, (b) 40 minutes, (c) 50 minutes, (d) 70 minutes, (e) 85 minutes and (f) 100 minutes.

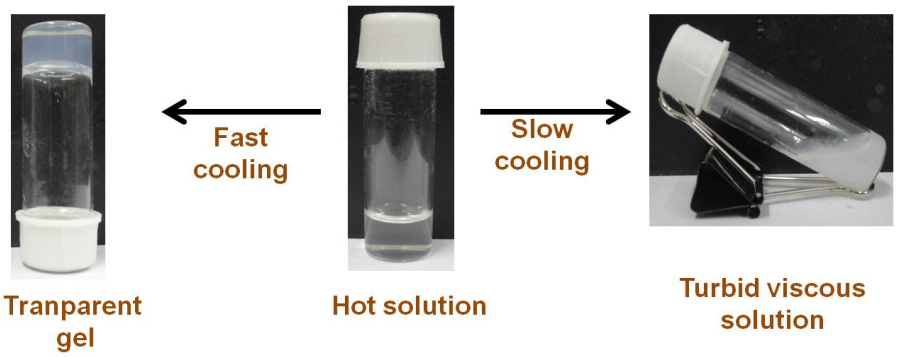

Fig. S5 Change of physical state of the gelator solution with change in the rate of cooling.

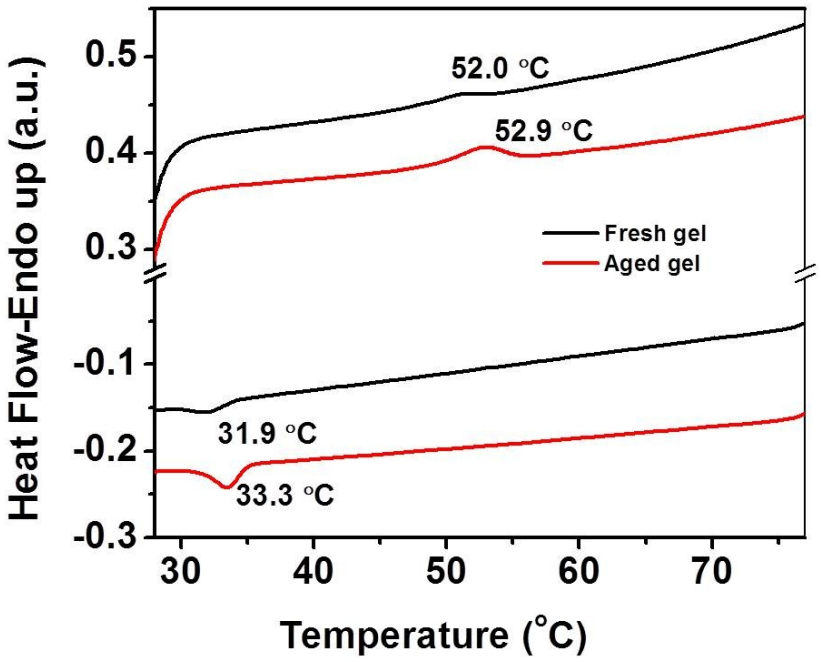

Fig. S6 Dynamic Scanning Calorimetry (DSC) analysis of the fresh and the aged gel.

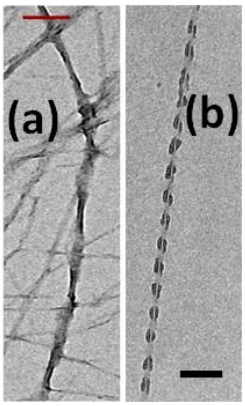

Fig. S7 A comparison between a single fiber obtained from fresh and aged **MAA** gel (scalebar 200 nm) confirming difference in pitch length of the helical fibres.

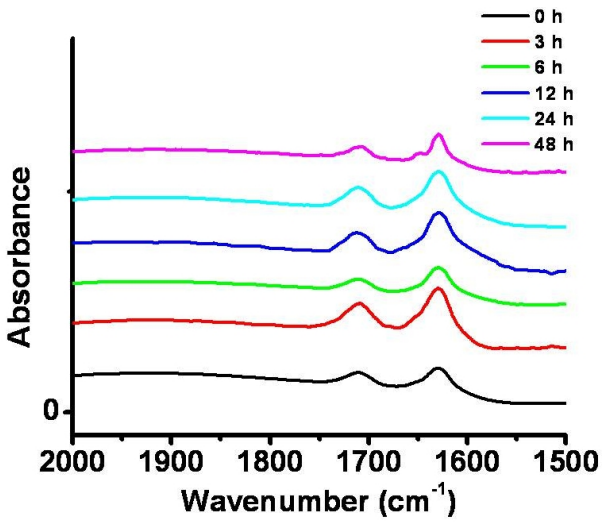

Fig. S8 Kinetic study of the D_2O gel of **MAA** with FTIR.

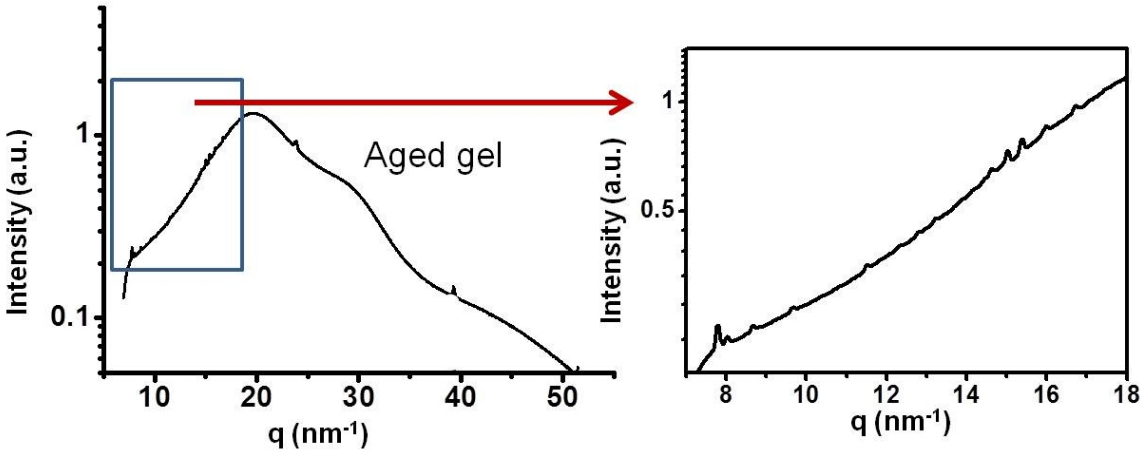

Fig. S9 WAXS of the aged/turbid **MAA** hydrogel.

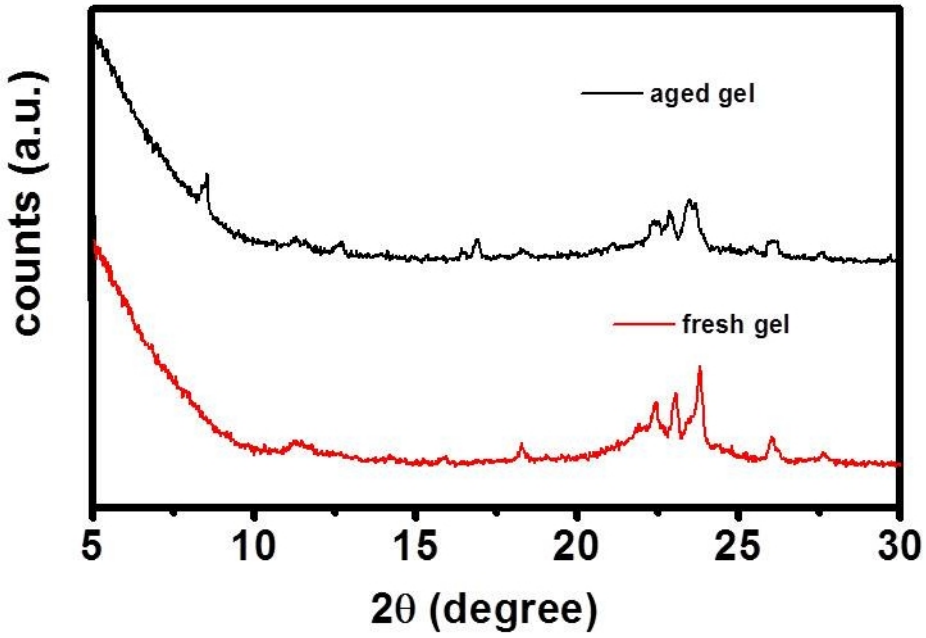

Fig. S10 Wide angle powder XRD of the **MAA** xerogel obtained from the aged and fresh gel.

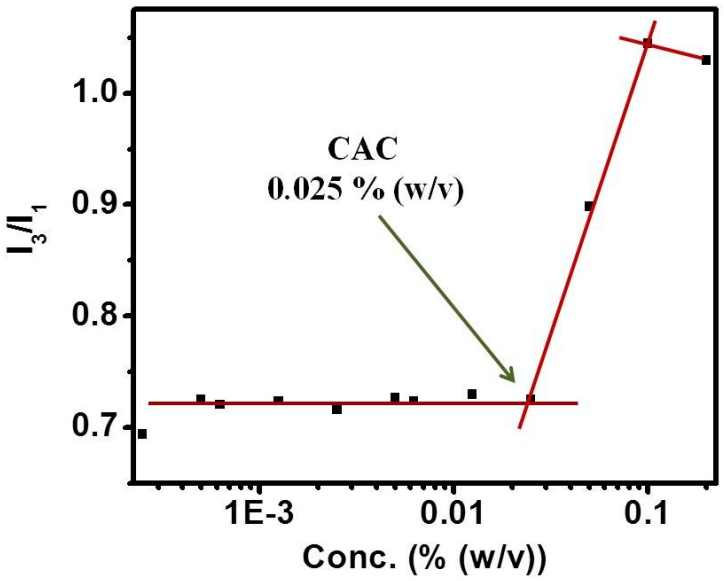

Fig. S11 Determination of critical aggregation concentration (CAC) of the gelator **MAA** with pyrene.

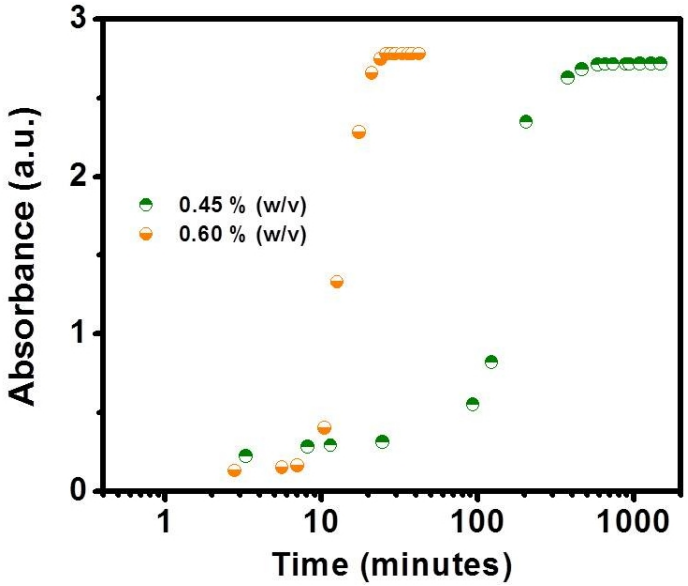

Fig. S12 Time-dependent absorbance studies of the hydrogel **MAA** at two different concentrations**.** Absorbance values are noted at 553 nm (λ_{max} of the reference dye Rhodamine B). It indicates a faster transparent-turbid transition for the concentrated hydrogel (0.6 % (w/v)) compared to the less concentrated hydrogel (0.45 % (w/v)).

Table S1 Peaks observed from SAXS and WAXS peaks obtained from **MAA** aged hydrogel.

q (nm^{-1})	d (Å)
1.48	42.45
1.91	32.89
7.79	8.06
8.04	7.81
8.64	7.27
9.66	6.50
11.52	5.45
12.31	5.10
12.78	4.92
13.22	4.75
14.64	4.29
15.02	4.18
15.39	4.08
15.69	4.0
16.72	3.76

Table S2 Peaks observed from low angle PXRD and wide angle PXRD peaks obtained from **MAA** aged xerogel.

2θ (degree)	d (Å)
2.18	40.48
2.69	32.80
8.58	10.29
11.30	7.82
12.70	6.96
16.47	5.37
16.94	5.23
18.30	4.84
21.16	4.19
22.40	3.96
22.86	3.88
23.47	3.78
25.38	3.50
26.12	3.41
27.55	3.23
29.72	3.00