Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Supplementary Information

Mechanoresponsive, Proteolytically stable and Biocompatible Supergelators from Ultra Short Enantiomeric Peptides with sustained drug release propensity

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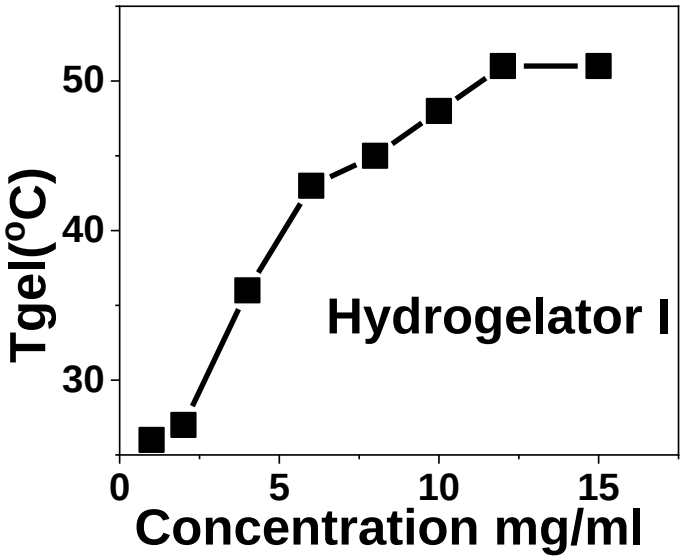

Figure S1A. Tgel graph of the Xerogel of Hydrogelator –**I**.

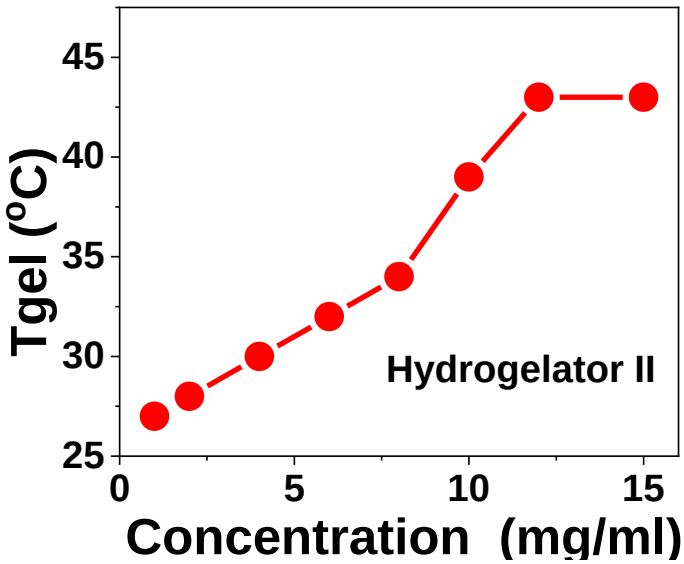

Figure S1B. Tgel graph of the Xerogel of Hydrogelator –**II**.

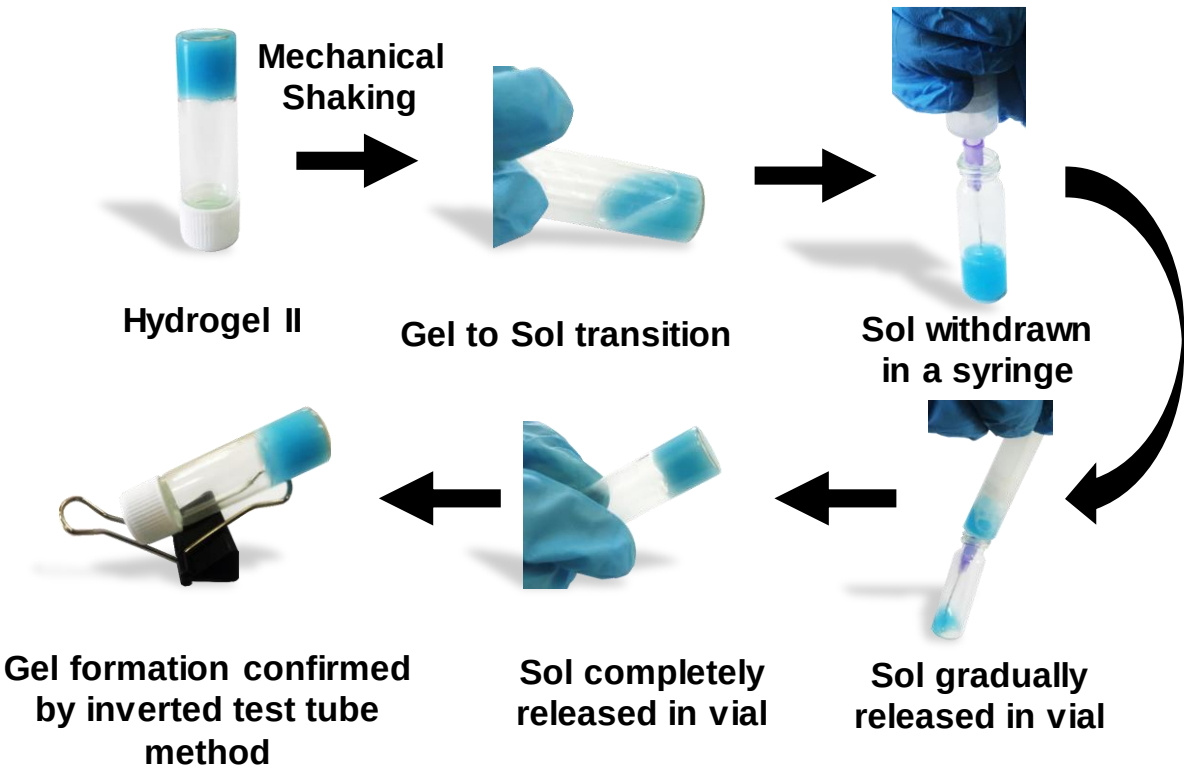

Figure S2. Demonstration of Thixotropic and Injectable property of Hydrogelator- **II**.

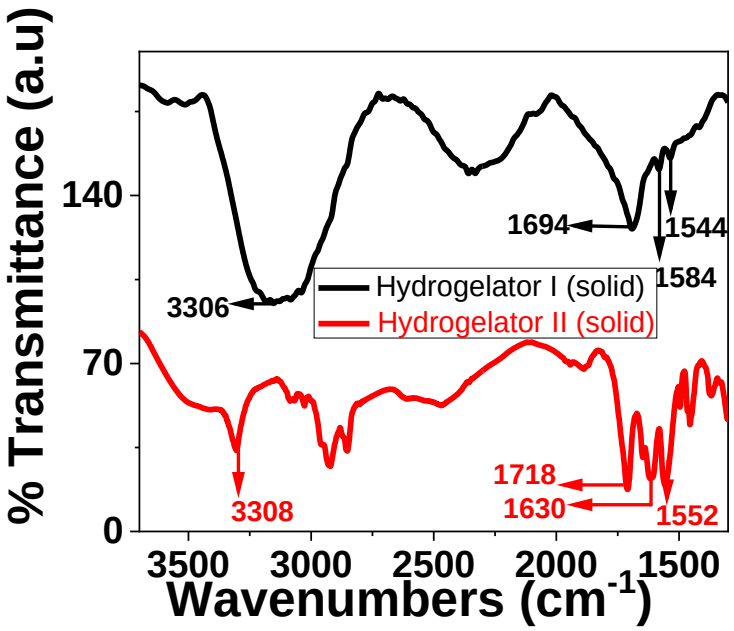

Figure S3A. Overlapped FT-IR Spectra of Hydrogelator I

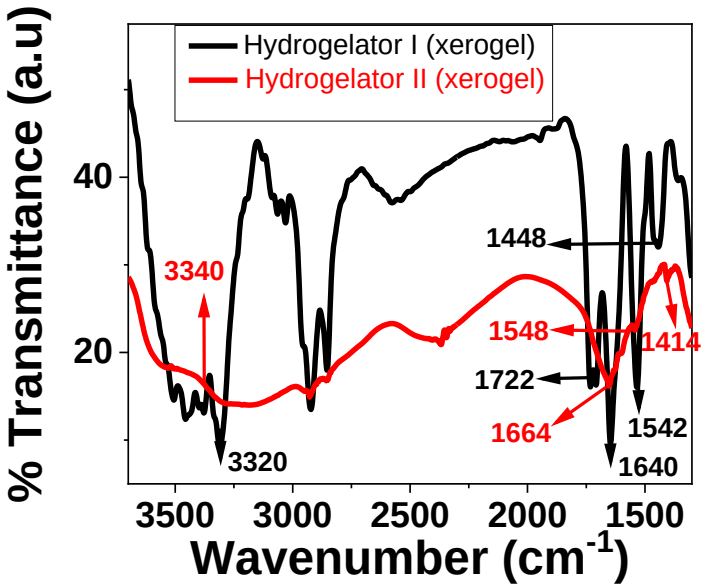

Figure S3B.Overlapped FT-IR spectra of Hydrogelator II

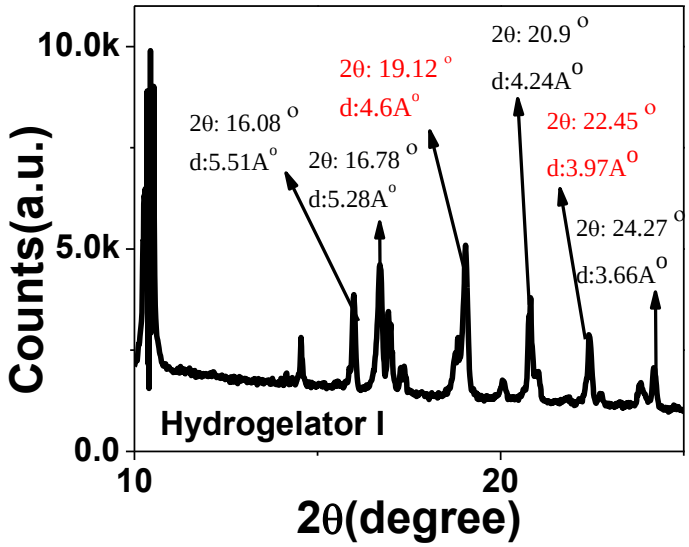

Figure S4A. PXRD spectrum for Hydrogelator I

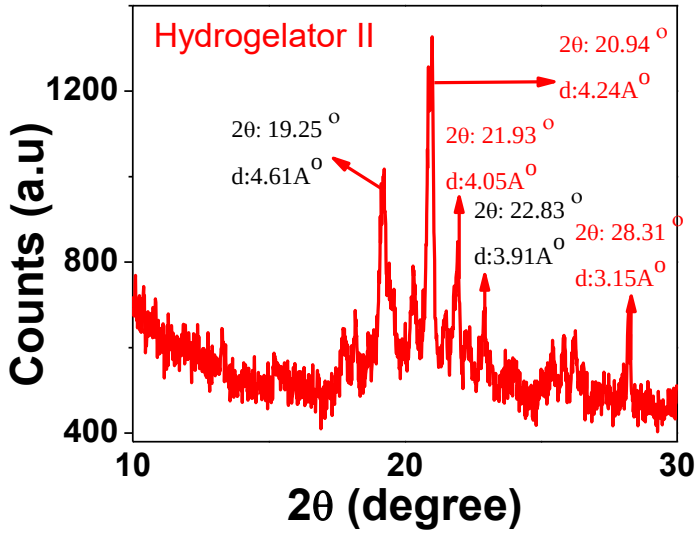

Figure S4B. PXRD spectrum of Hydrogelator II

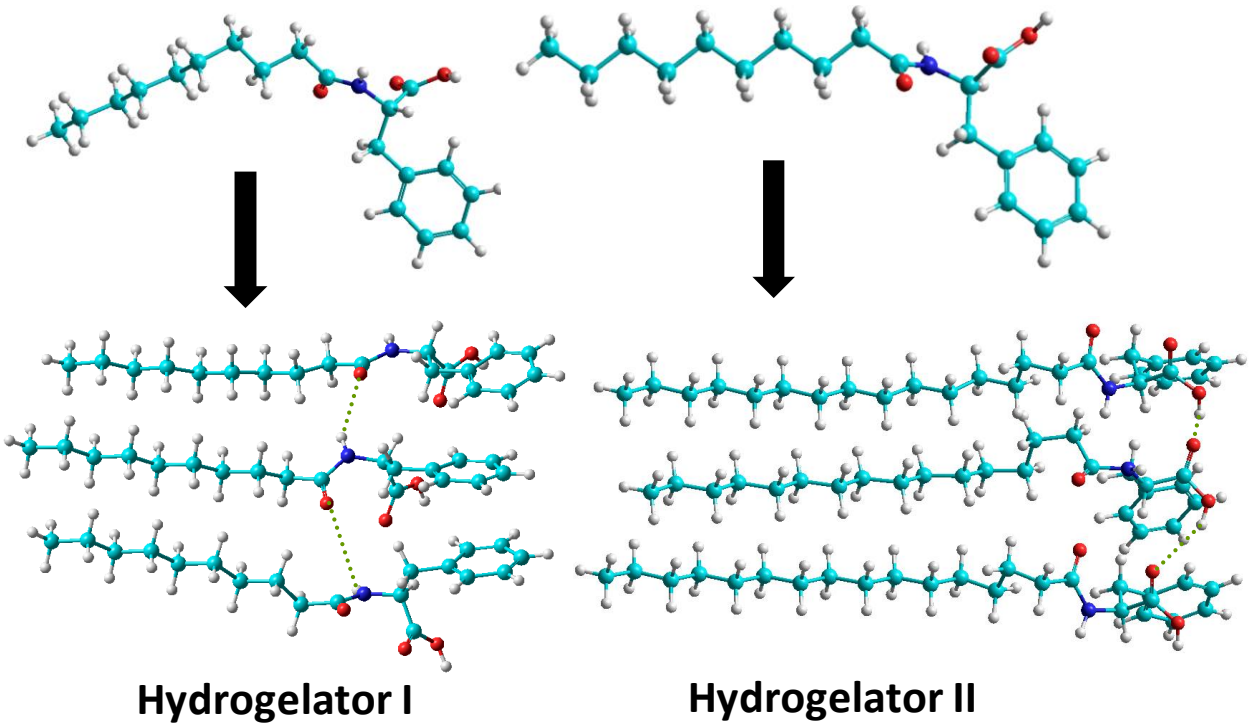

Figure S5. Energy Optimized Structures of Hydrogelator **I (A) – II (B)** forming β-sheet like assembly, stabilized by non-covalent interactions.

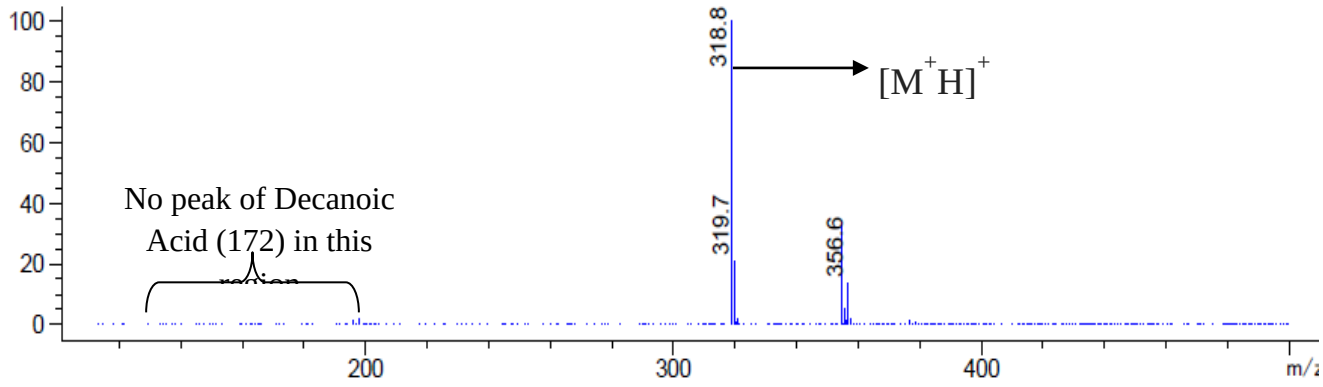

Figure S6A: Mass spectrum of Hydrogelator - **I** before incubation with Proteinase K.

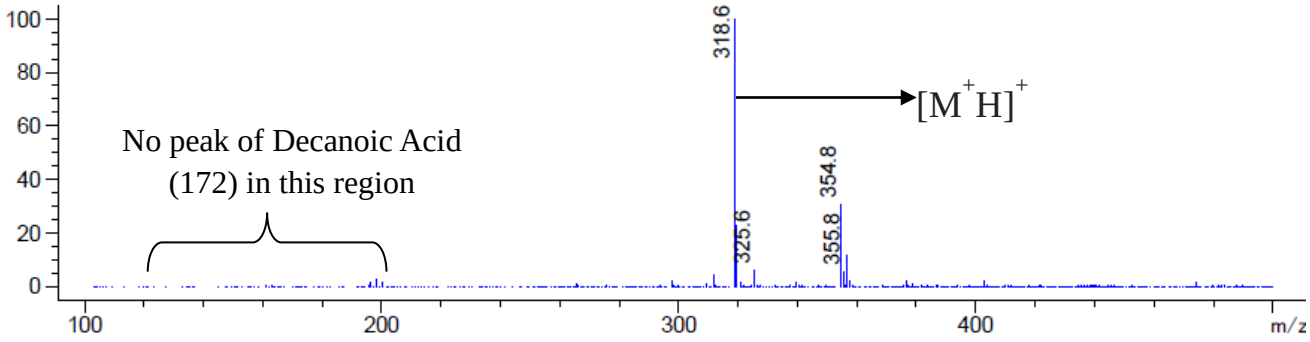

Figure S6B: Mass spectrum of Hydrogelator - **I** after 24 hours incubation with Proteinase K.

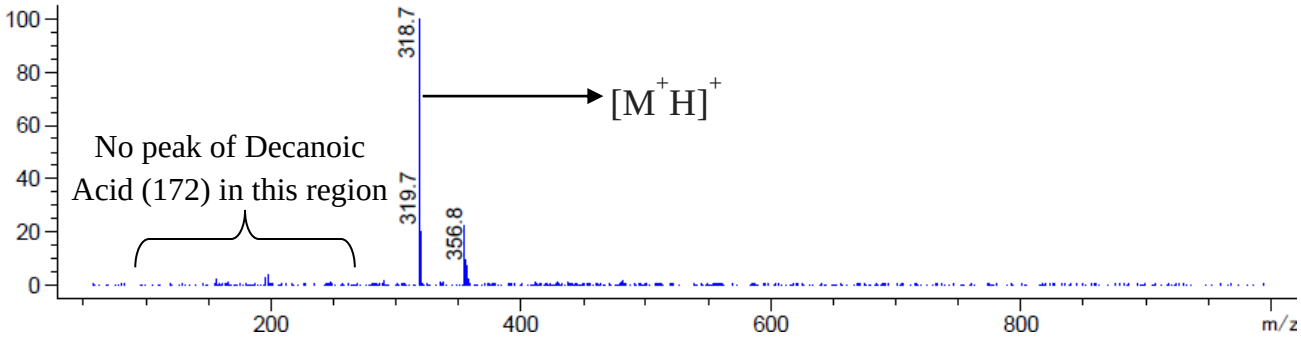

Figure S6C: Mass spectrum of Hydrogelator - **I** after 48 hours of incubation with Proteinase K.

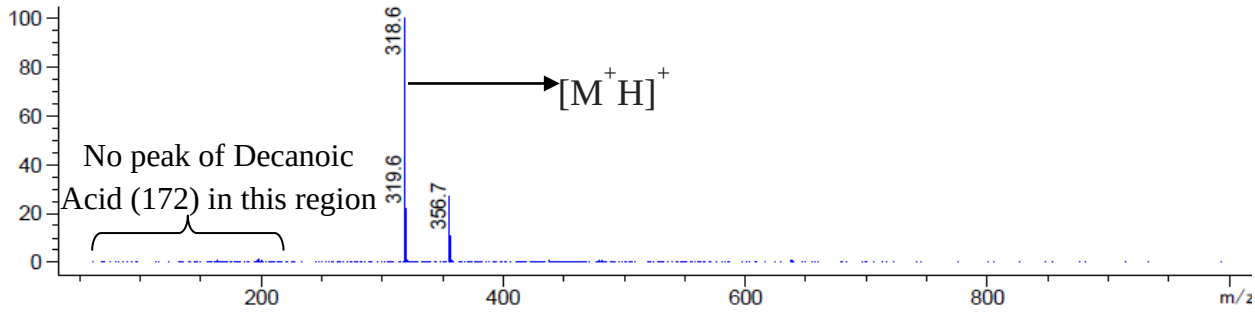

Figure S7A: Mass spectrum of Hydrogelator - **II** before incubation with Proteinase K.

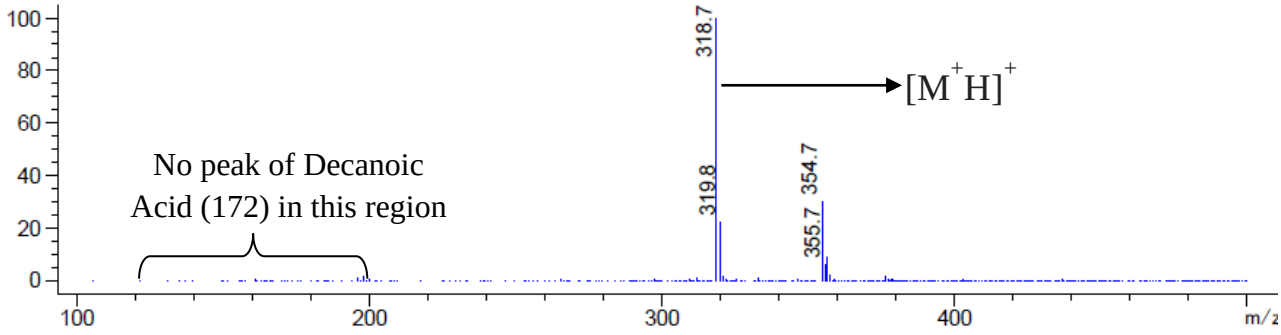

Figure S7B: Mass spectrum of Hydrogelator - **II** after 24 hours incubation with Proteinase K.

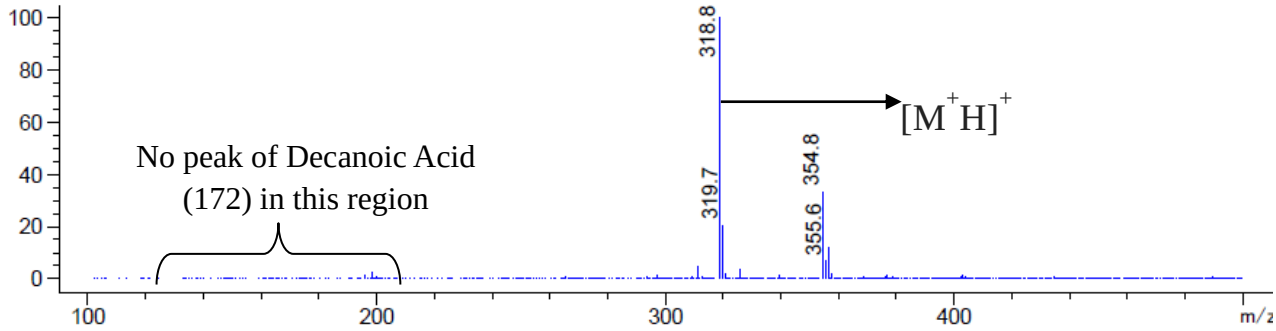

Figure S7C: Mass spectrum of Hydrogelator -**II** after 48 hours of incubation with proteinase K.

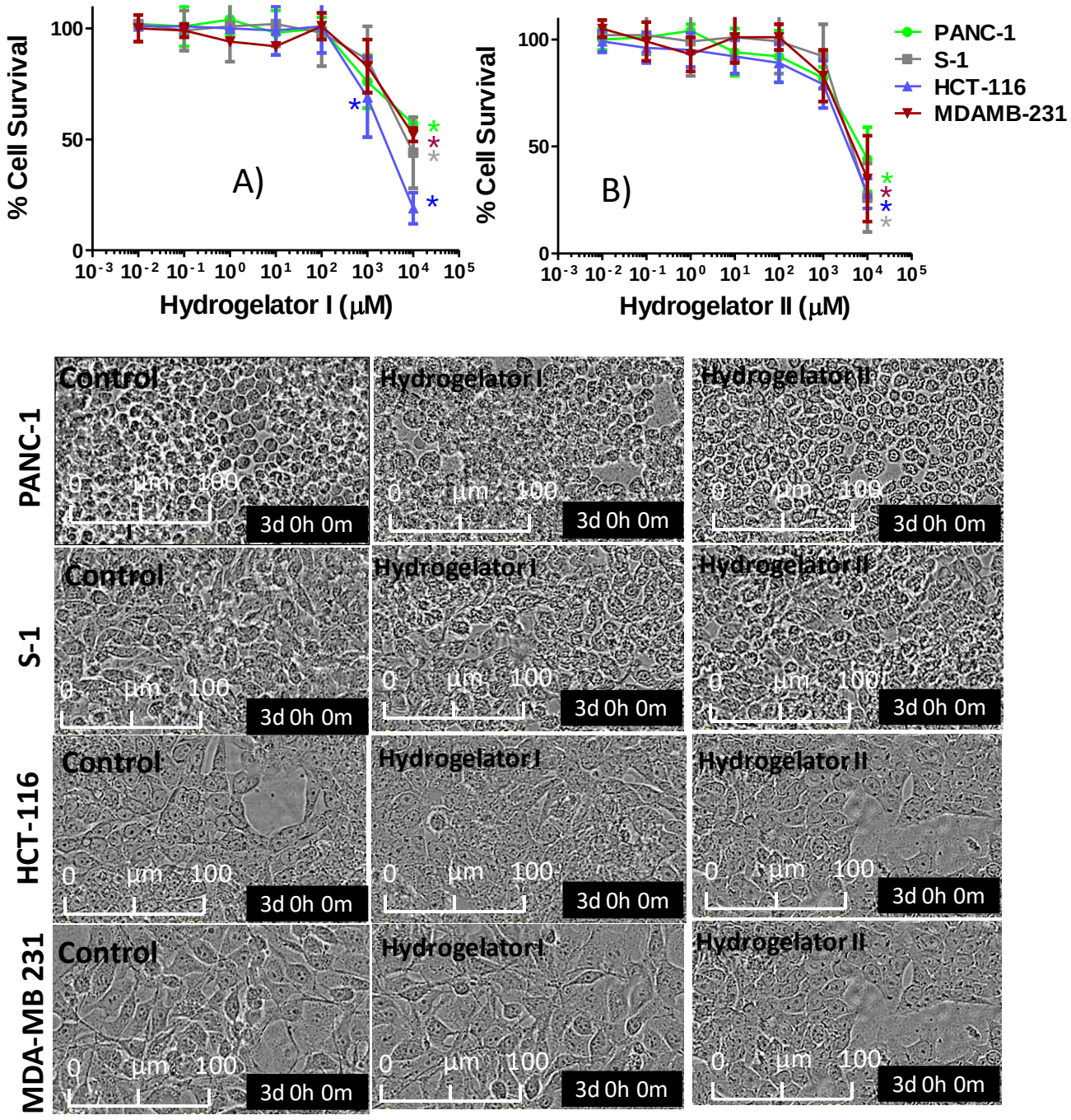

Figure S8: Enlarged view of the morphological study of cell lines after treatment with the Hydrogelators.

Experimental

Preparation of the Hydrogel: For preparation of the hydrogels, 1 mg of each of the hydrogelators were dissolved in 1ml of phosphate buffer in glass vials by heating on a hot plate to obtain a homogenous mixture. These solutions were then slowly cooled to room temperature (20^0C) and kept undisturbed for the gelation studies. The formation of the hydrogel was confirmed by the inverted vial method.

Rheological Properties of the Hydrogels. Rheological experiments were performed at 25 °C on an Anton Paar Physica MCR 301 rheometer. The viscoelastic properties of hydrogels were measured by measuring the storage modulus (G') and loss modulus (G''). Hydrogel (1 mL) was transferred on a rheometer plate by using a microspatula and kept hydrated by using a solvent trap. A stainless steel parallel plate (diameter: 25 mm) was used to sandwich the hydrogels with TruGap (0.5 mm). The dynamic strain sweep experiment was performed to determine the region of deformation of hydrogels in which linear viscoelasticity is valid. The exact strains for hydrogel materials were determined by linear viscoelastic regime at a constant frequency of 10 rad s−1. The mechanical strengths of the hydrogels were determined by frequency sweep experiment. In the frequency sweep measurement, the graph was plotted as a function of frequency in the range of 0.05−100 rad s^{-1}. The thixotropic properties were investigated by step-strain experiments at the constant frequency of 10 rad s^{-1}, and applied strains were varied from 0.1 to 40%.

Circular Dichroism (CD) Spectroscopy. CD measurements of all peptide hydrogels were performed using a Jasco J-1500 CD spectrophotometer (Easton MD, USA). A 0.1 mm quartz cuvette was used in which three repeat scans were compiled to generate average spectra at a scanning rate of 200 nm/min. The results were analyzed using Jasco spectra manager.

Fourier-transform infrared (FTIR) Spectroscopy. FTIR spectra were recorded using a KBr pellet method. A freeze-dried sample of the gel was mixed with KBr to make the pellet. The spectrum was acquired from 400 to 4000 cm^{-1}, and 20 accumulations were averaged to obtain single spectra on an Agilent CARY 620 FTIR spectrophotometer. The background was collected using a blank KBr pellet. Spectra were background subtracted to correct for atmospheric interference.

Powder X-ray diffraction (PXRD) study

The hydrogel was freeze dried by using liquid nitrogen at first and it was further dried in lyophilizer to get xerogel for X-ray diffraction study. The dried powder was then placed on a glass plate and the experiment was carried out by using an X-ray diffractometer (Bruker AXS, Model No. D8Advance). The instrument was operated at 40 kV voltages and 40 mA

current and was calibrated with a standard Al_2O_3 (corundum) sample before use. For scan, the Lynx Eye superspeed detector was used with scan speed 0.3 s and step size 0.028.

Theoretical Studies

The target molecules were first geometry optimized using Hyperchem Trial Version 8.01 (Hypercube Inc., USA). All calculations related to geometry optimization of the molecular structures were done with AMBER 99 molecular mechanics Force-Field. Individual molecules were first geometry optimised which were then used for determining the structures of the subsequent multi-molecular systems. Conformational search for the geometry optimization studies were done using Polak-Ribiere (conjugate gradient) algorithm with RMS gradient of 0.01 kcal/(Å mol). To further understand the energetics of the hydrogelator systems in particular, the geometry optimised structures as obtained above, were further subjected to a single-point energy calculation using PM3 semi-empirical algorithm.

Field Emission scanning electron microscopic study (FESEM)

The morphology of the xerogels obtained from dipeptide derivatives **I** and **II** were gold coated and observed under a FESEM microscope (JEOL JSM - 6700F).

MTT Viability Assay. Cells (5 × 104) were seeded into 96-well plates in 198 μL of Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS), and 2 μL of initial hydrogel concentrations (ranging from 20 to 0.031 mM) was added into wells (final concentrations were reported in the figures after dilutions). Only the medium containing FBS was used as a blank, whereas the control was containing cells in media along with 2 μL of buffer. Top five higher concentrations (5, 7.5, 10, 15, and 20 mM) of both the hydrogels were also used for the MTT test. hWBCs were grown and mixed with 50, 75, 100, 150, and 200 μL of hydrogels, and DMEM was used to make up the total volume to 200 μL. The cells were directly mixed in hydrogels (20 mM) and incubated for 24 h for the biosafety assessment of the hydrogels. For each group, eight wells were assigned and plates were kept for 24 h in a 5% CO2 incubator. After completion of incubation, 5 μg of the final concentration of MTT (thiazolyl blue tetrazolium bromide, from Sigma-Aldrich, MO, USA) reagent (20 μL from 5 mg/mL stock) was used in each well. After 3 h incubation at 37 °C in the 5% CO2 incubator, 100 μL of dimethyl sulfoxide (DMSO) and ethanol (1:1) mixture was added into each well. Absorbance was taken by the microplate reader at 450 nm against blank.

Synthesis of HNPs

The hydrogel nanoparticles (HNPs) were prepared using self-assembly and modified inverse emulsion technique in which vitamin E-TPGS was used as an emulsion stabilizer. In short, HNPs were formulated by the dilution of stock solution (100 mg/mL) in pure

watej to a final peptide concentration of 10 mg/mL. The resulting solution was added drop-wise into 50 mL slightly warmed mineral oil containing vitamin E-TPGS at concentration of 0.4% wt/v and homogenized using high speed homogenizer. Next, nanoparticles were allowed to self-assemble for 2 h with continuous stirring at 4ºC to allow the surfactant monolayer accumulation on the surface of the nanoparticle. Upon the completion of the self-assembly process, the resulting suspension was mixed with a non-polar solvent, and centrifuged to obtain phase separation. Supernatant was removed and HNPs were washed again to remove the remaining residues of mineral oil and finally vacuum dried. The obtained HNPs were used immediately, or stored at 4ºC for later use.

Nanoparticle characterization

Nanoparticle size diameter and surface charge were measured using Malvern Zeta-sizer, with 4mW 633 He-Ne Laser, (DTS version 4.10, Malvern, U. K.) with appropriate viscosity and refractive index settings. The temperature was maintained at 25^{o}C during the measurement.

In vitro drug release of drugs from HNPs

The in vitro release profile of the model drugs 5FU and doxorubicin from the drug loaded HNPs were performed using dialysis membrane previously soaked for 24 hrs in the dissolution membrane and stretched around at one end of the tube. The drug loaded formulations were carried out using pretreated membrane which were immersed into 30 mL of phosphate buffer solution of pH 7.4 for both the drugs at room temperature and magnetically stirred at 50 rpm. At selected time intervals aliquots were withdrawn from release medium and replaced with same amount of phosphate buffer (1 ml). The samples were analyzed thrice using UV-spectrophotometer at 267nm for 5FU and 495nm for doxorubicin. The percentage of cumulative drug release was plotted against time to obtain the release curves.

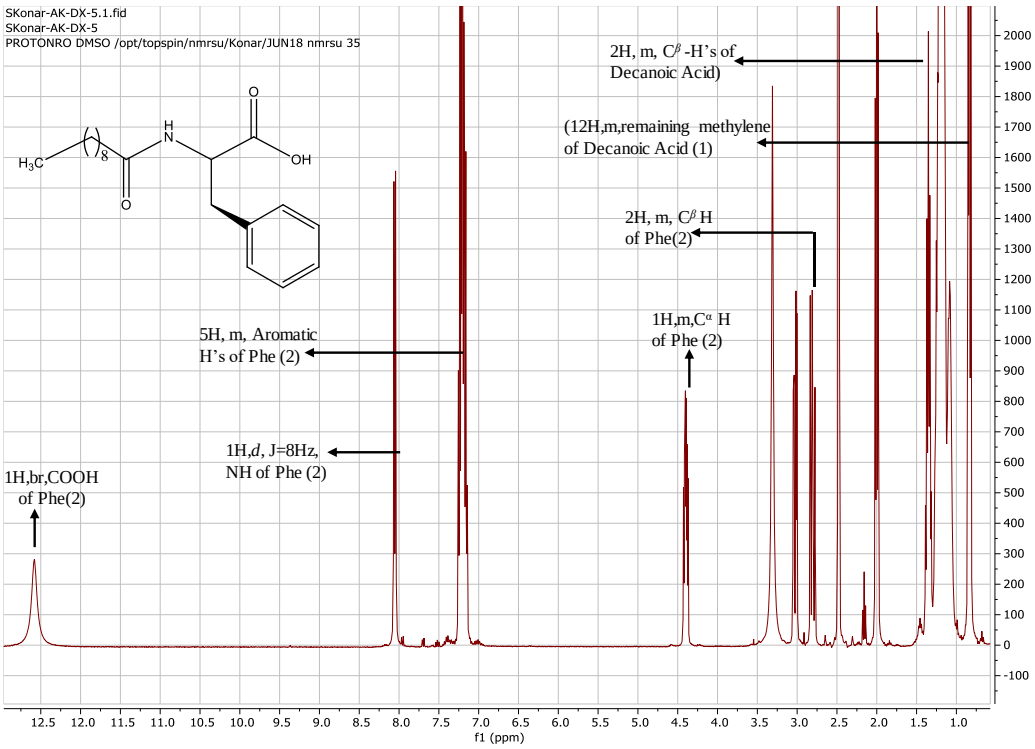

Figure S9A.^{1}H NMR Spectrum of Hydrogelator-**I**

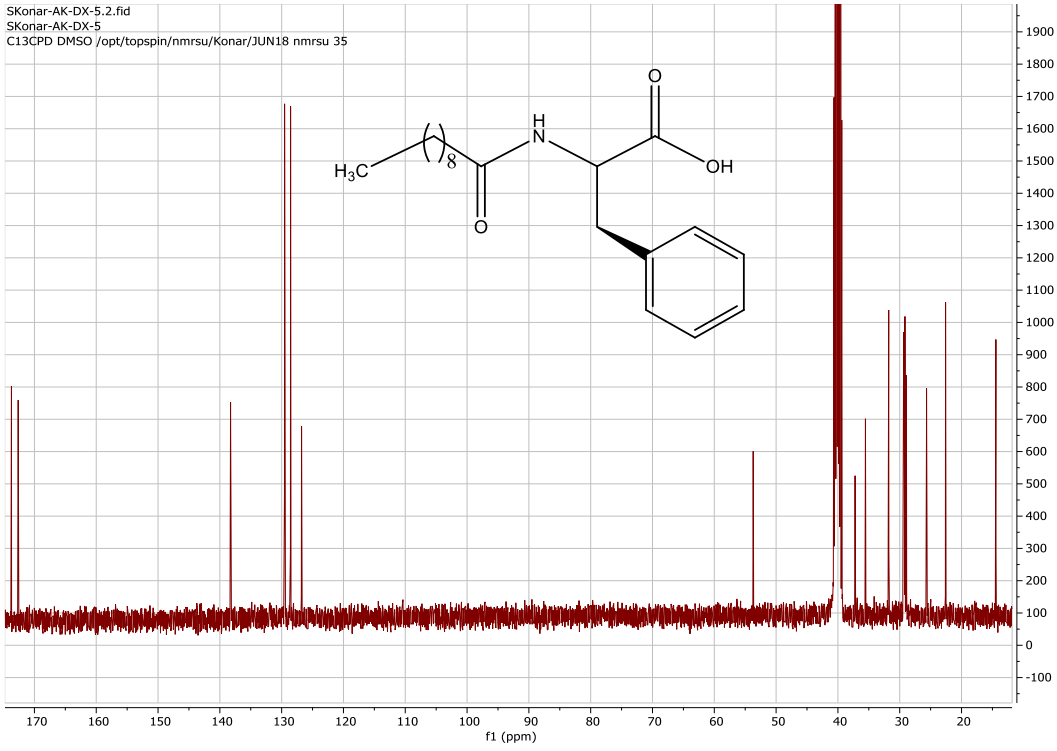

Figure S9B.^{13}C NMR Spectrum of Hydrogelator-**I**.

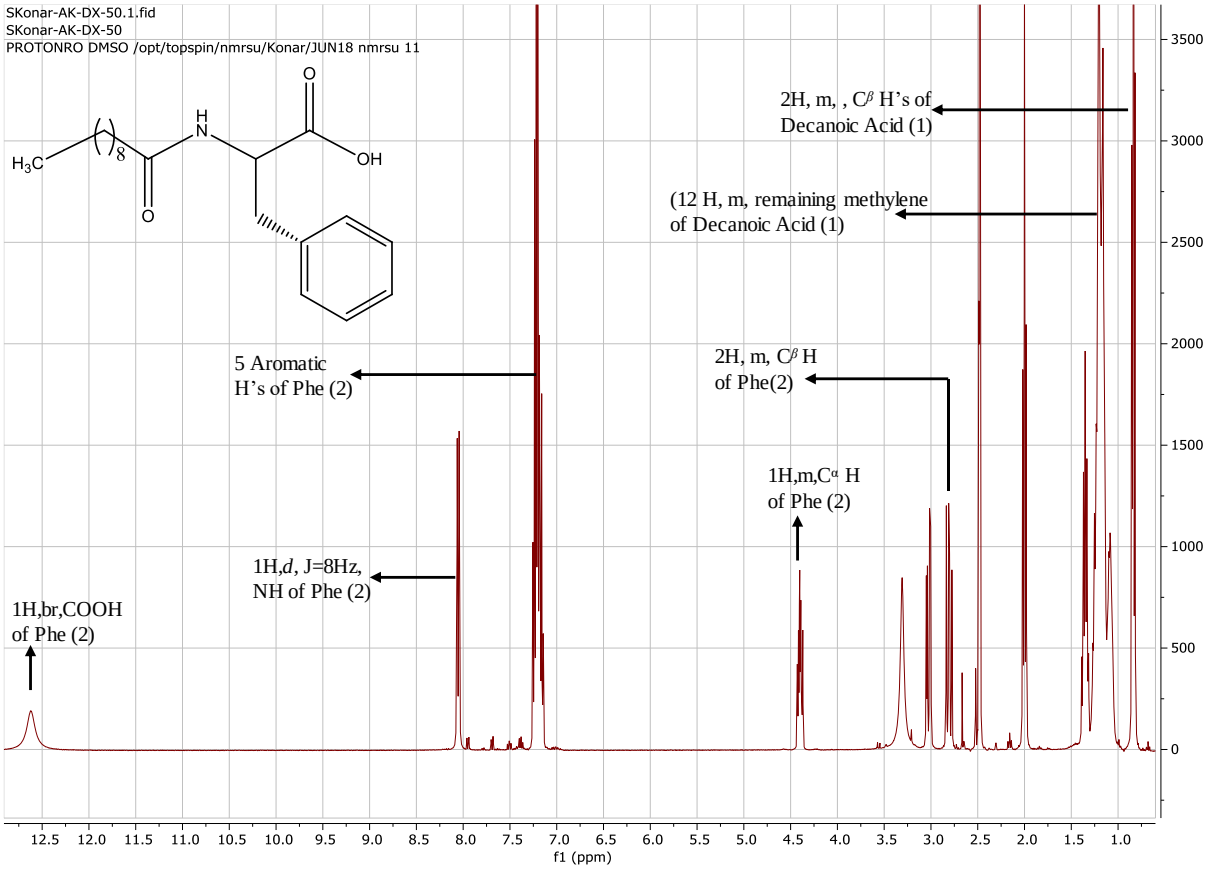

Figure S10A.[1]H NMR Spectrum of Hydrogelator-**II**.

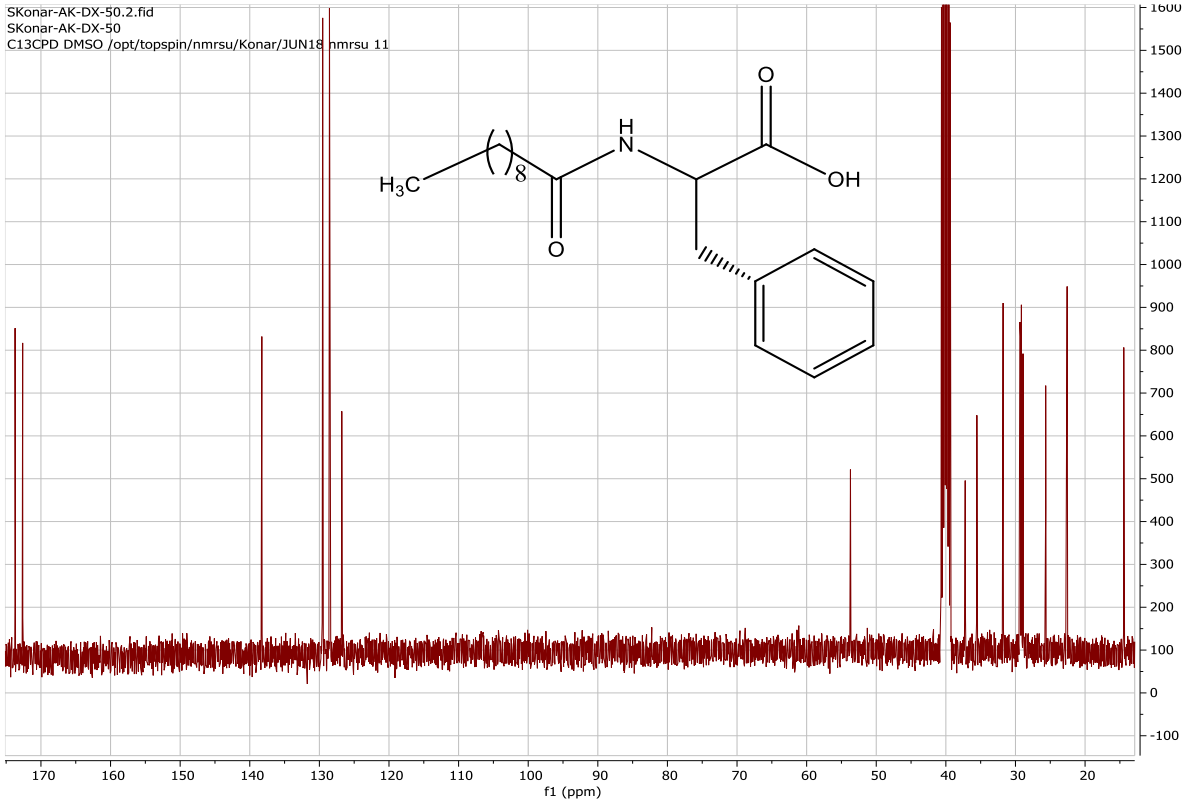

Figure S10B.[13]C NMR Spectrum of Hydrogelator-**II**.