Supporting Information

for

Tuning Viscoelastic Properties of Supramolecular Peptide Gels via Dynamic Covalent Crosslinking

Experimental Section

Materials

9-Fluorenylmethoxycarbonyl protected amino acids, MBHA Rink Amide Resin, HBTU (O-Benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate)are purchased from Novabiochem, lauric acid is purchased from Merck. Glutaraldehyde (25%)was purchased from Merck. All chemicals were used directly without any further purification.

Peptide Synthesis

In the synthesis of peptide amphiphile, solid phase peptide synthesis method was applied with an automated peptide synthesizer (CS Bio. Company model:136XT). Peptides were constructed on MBHA Rink Amide resin. Amino acid couplings were done with 2 equivalents of fluorenylmethyloxycarbonyl (Fmoc) protected amino acid, 1.95 equivalents O-Benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate (HBTU) and 3 equivalents of N,N-diisopropylethylamine (DIEA) for 3 h. Fmoc removals were performed with 20% piperidine /dimethylformamide solution for 10 min. Cleavage of the peptides from the resin was carried out with a mixture of trifluoroacetic acid:triisopropylsilane:water in ratio of 95:2.5:2.5 for 3 h. Excess trifluoroacetic acid was removed by rotary evaporation. The remaining viscous peptide solution was triturated with cold ether and the resulting white product was lyophilized.

Liquid Chromatography

For the structural analysis of the peptide Agilent Technologies 6530 Accurate-Mass Q-TOF LC-MS and Zorbax SB-C8 column were used. Concentration of the sample for LC-MS measurement was 0.5 mg/ml. Solvents were water (0.1% formic acid) and acetonitrile (ACN) (0.1% formic acid). LC-MS was run for 25 min for each sample and it started with 2% ACN and 98% H_2O for 5 minutes. Then, ACN gradient reached to 100% until 20 minutes. Finally, its concentration was

dropped to 2% and it kept running for 5 minutes. Solvent flow 0.65 mL/min and 5 µL sample was injected. The synthesized peptide amphiphile was further purified by prep-HPLC.

Rheology

Anton Paar MCR-301 rheometer was used for mechanical characterization of peptide amphiphile gels. Solutions of K2-PA (1% w/v, 2% w/v) were prepared at pH 7.5and gel formation was achieved with the addition of NaOH (1 M). Glutaraldehyde (10 µl) was used for crosslinking of peptide chains. Prior to rheology analysis samples were kept at 4°C for a total of 1 h. Crosslinked samples were kept at 4°C for 30 min after NaOH addition samples and for an additional 30 min after glutaraldehyde addition. Rheology measurements of different mixtures were performed to understand the mechanical properties of the resulting gels. Mechanical characterizations were accomplished using Anton Paar MCR-301 rheometer. Total volumes of the samples were 300 µL and during the analysis, PP25-SN17979 measuring device with 25 mm diameter was used. Measuring distance was determined as 0.5 mm. Time sweep tests of each sample were carried out for 1 hAngular frequency and strain magnitudes were determined as ω=10 rad/s and γ=0.1%, respectively. Angular frequency tests was carried out within logarithmically ramped range from ω=0.1 to 100 rad/s, during strain was kept constant at γ=0.1%, which is within the linear viscoelastic regime. Strain sweep tests was carried out within logarithmically ramped range from γ =0.01 to 500, during frequency was kept constant at ω=10 rad/s. Thixotropic behavior was investigated as time-dependent recovery after high shear load. In the first part of the experiment, gels at equilibrium modulus were deformed in LVR, 0.01% for 3 min. Then, strain was logarithmically ramped to 1000% within 1 min followed by recovery of deformation back again in the LVR, at 0.01% for 10 min. During thixotropic analyses, angular frequency was held constant at 10 rad s^{-1}.

Circular Dichroism (CD) Spectroscopy

Secondary structure of the peptide amphiphile was analyzed with JascoJ-815 circular dischroism spectrometer. 1 wt% peptide solution was prepared in ddH_2O then aged overnight. Peptide solutions were gelified with 1 M NaOH. After 1h peptide gels were diluted with dd H_2O.4.26x10^{-5} M peptide solutions were measured from 300 nm to 180 nm with 0.1 data pitch,

100 nm/min scanning speed, 1 nm band width and 1 s D.I.T. Average of two measurements were used and sensitivity was selected as standard.

UV-vis Spectroscopy

Cary 100 Bio UV-visible Spectrophotometerwas used to measure the absorbance of the samples.1 wt% peptide solution was prepared in ddH_2O then aged overnight. Peptide solutions were gelled with 1 M NaOH. After 1h, peptide gels were diluted with dd H_2O and $2.13x10^{-4}$ M peptide solutions were measured from 800 nm to 200 nm.

Scanning Electron Microscopy/Critical Point Dryer

FEI Quanta 200 FEG environmental scanning electron microscope (SEM) was used to image peptide amphiphile gel after removing solvent with Tousimis Autosamdri-815B, Series C critical point dryer (CPD). 1 wt% solution of peptide in distilled water was prepared and 1 M NaOH solution was added to peptide solution on metal mesh to adjust the pH around 7.5. Since critical point dryer can be used with samples in ethanol, we washed peptide gels with 20%, 40%, 60%, 80% and 100% (v/v) ethanol solutions. Then, gels were dried with a critical point dryer. Finally, peptide amphiphile network was imaged with SEM.

Transmission Electron Microscopy

FEI Tecnai G2 F30 transmission electron microscope (TEM) was used for imaging. 1 wt% peptide solution was prepared in ddH_2O then aged overnight. Peptide solutions were gelled with 1 M NaOH. After 1h, peptide gels were diluted 200 times with dd H_2O. Small amount of solution was dropped to carbon covered copper grid and 2% (w/v) uranyl acetate solution was used for staining. Finally, carbon grid was dried at atmosphere for 3h.

Zeta potential and pH analysis

Zeta potential measurements were performed with Malvern Nano-ZS Zetasizer equipped with a titrator and pH meter.0.02 wt% peptide solutions were prepared by dissolving 3mg of peptide in 15 ml of dd water. Smoluchovski approach was used to calculate the Zeta Potential values. The pH was measured by addition of 5ul of 0.1 M NaOH to the peptide solutions.

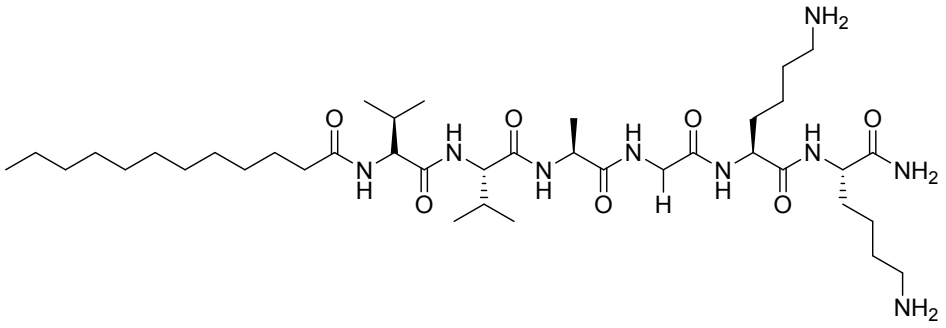

Figure S1. Chemical structure of Lauryl-VVAGKK-Am peptide amphiphile molecule

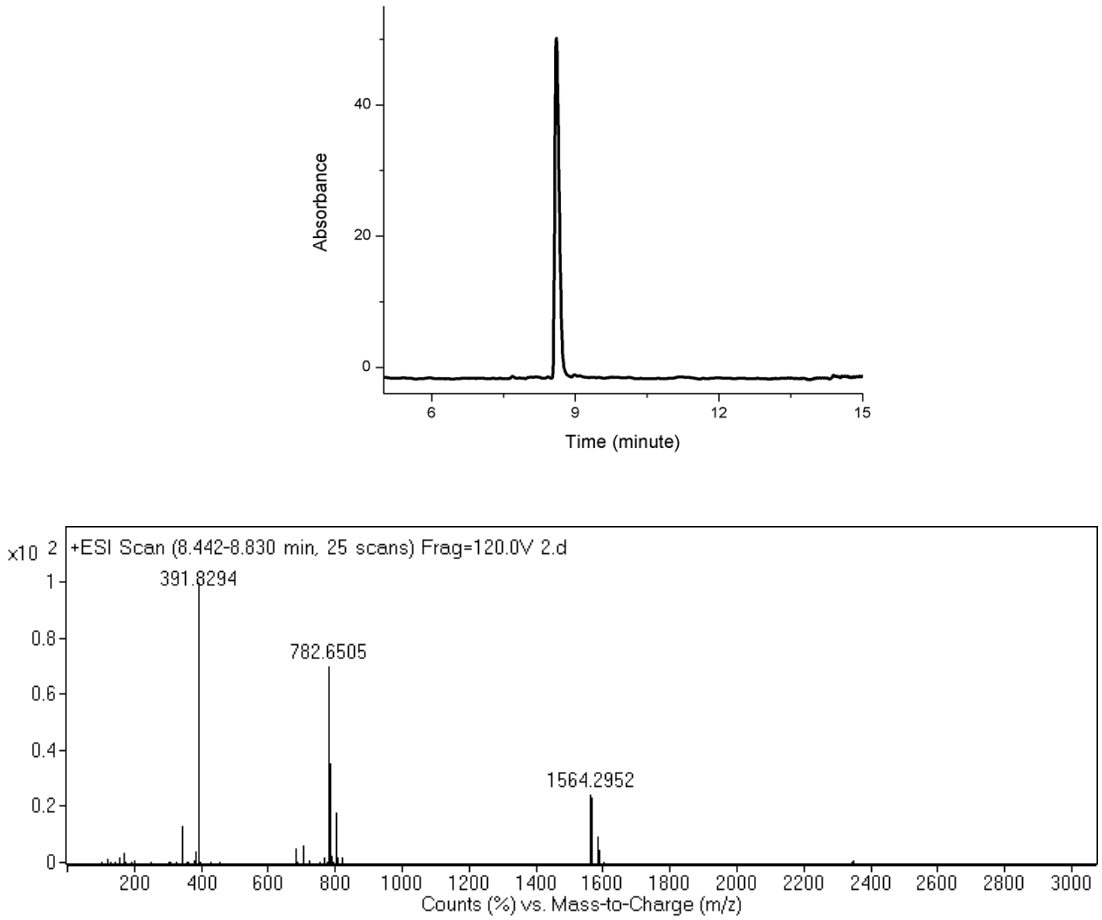

Figure S2.HPLC chromatogram of peptide.Absorbance at 220 nm vs retention time graph (top).Mass spectrometry of peptide after substracting mass spectra of water sample at that time interval (bottom). [M+H]+(calculated)=782,02, [M+H]+(observed)=782.65, [M+2H/2]+(calculated)=391.01, [M+2H/2]+(observed)=391.82.

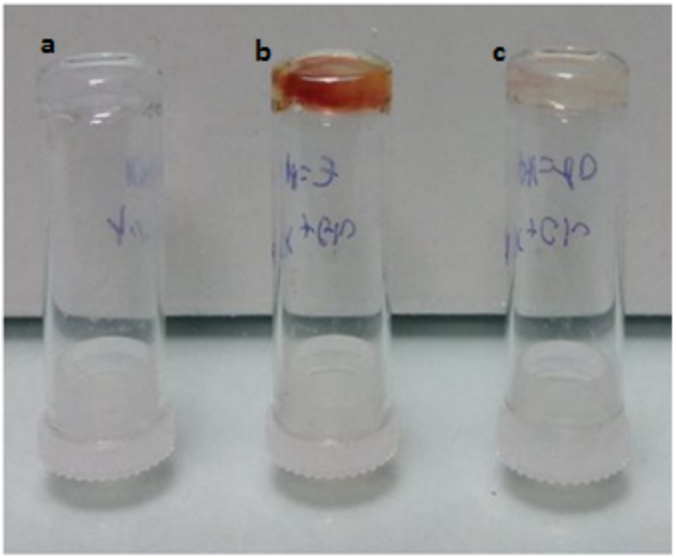

Figure S3. Peptide gels (a) before crosslinking, (b) Crosslinked at pH7.5, and (c) Crosslinked at pH10.

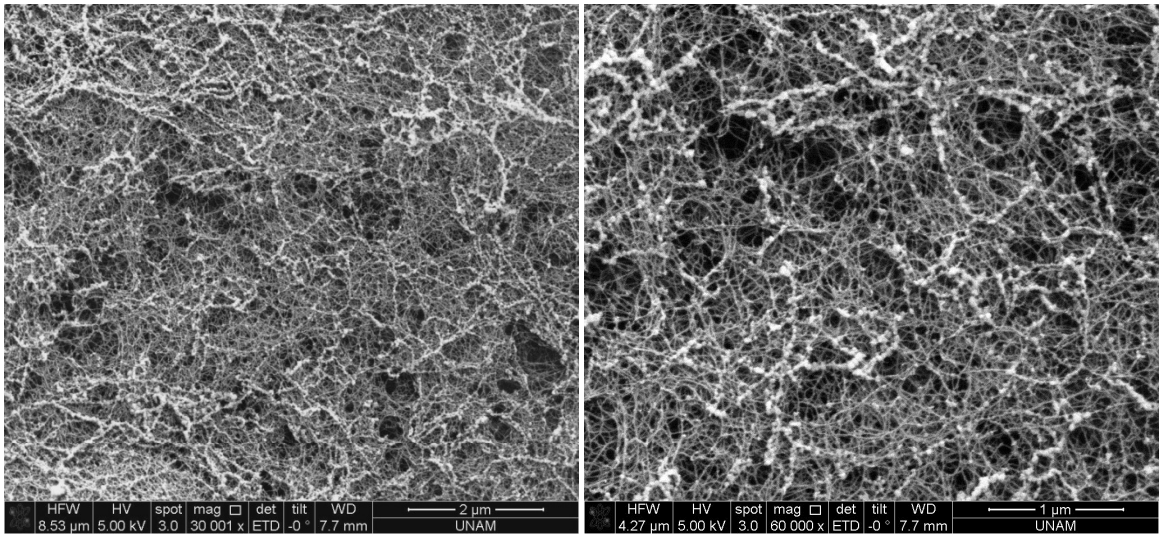

Figure S4. 2 wt% peptide gels crosslinked by glutaraldehyde at pH 7.5.

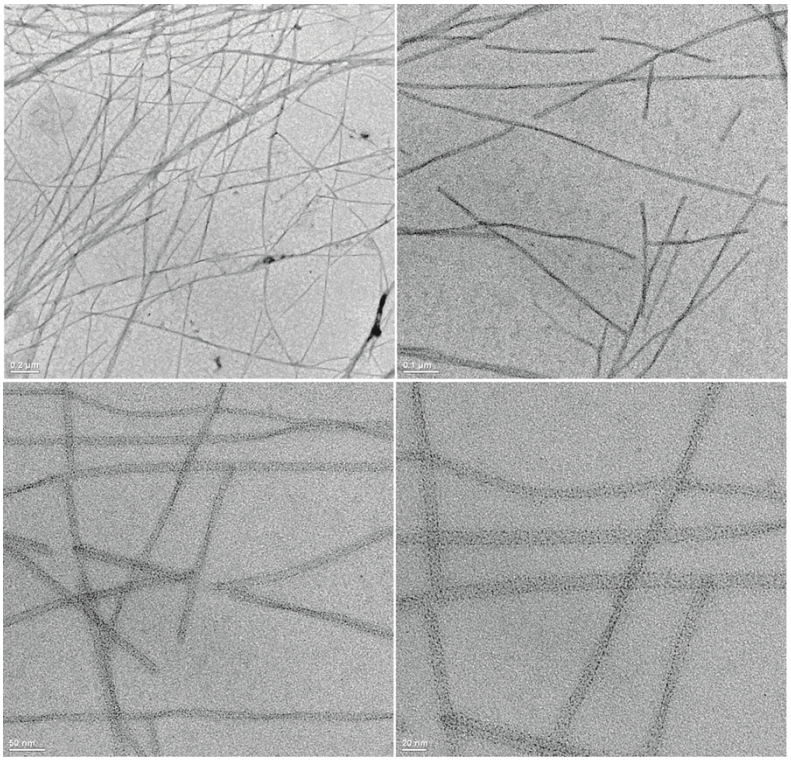

Figure S5. TEM images of peptide nanofibers at pH 7.5

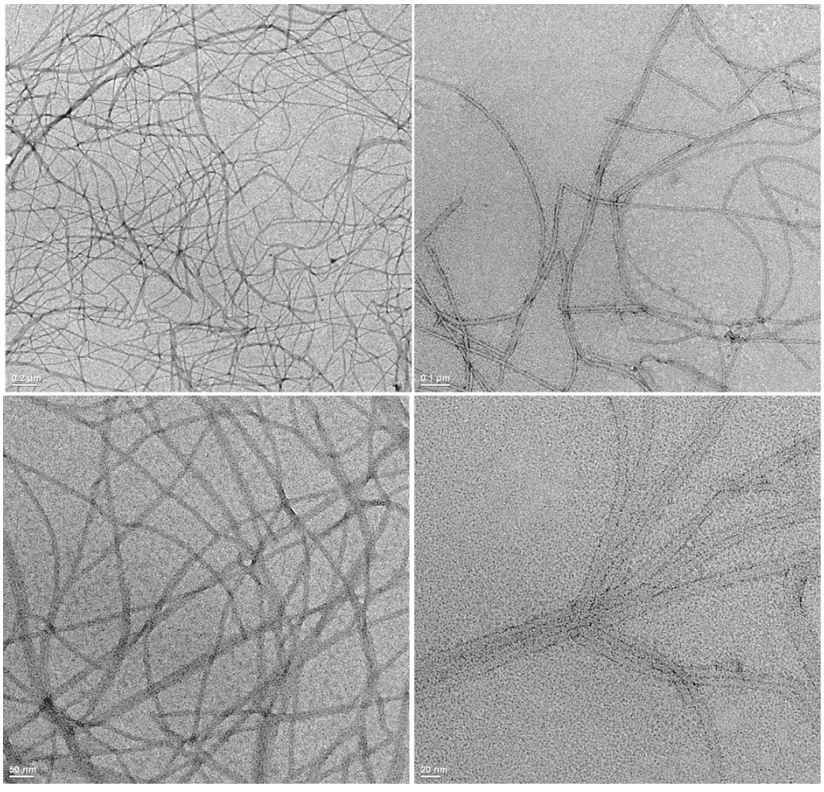

Figure S6. TEM images of gels crosslinked by glutaraldehyde at pH 7.5

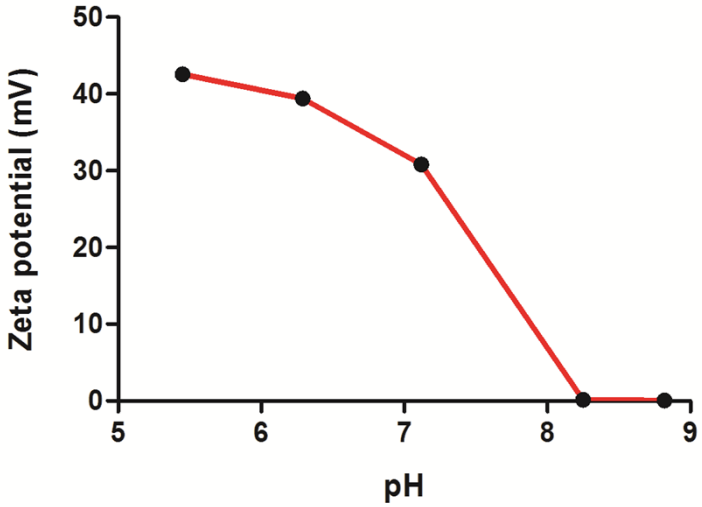

Figure S7. pH measurement of peptide amphiphile molecules by 0.1M NaOH addition.

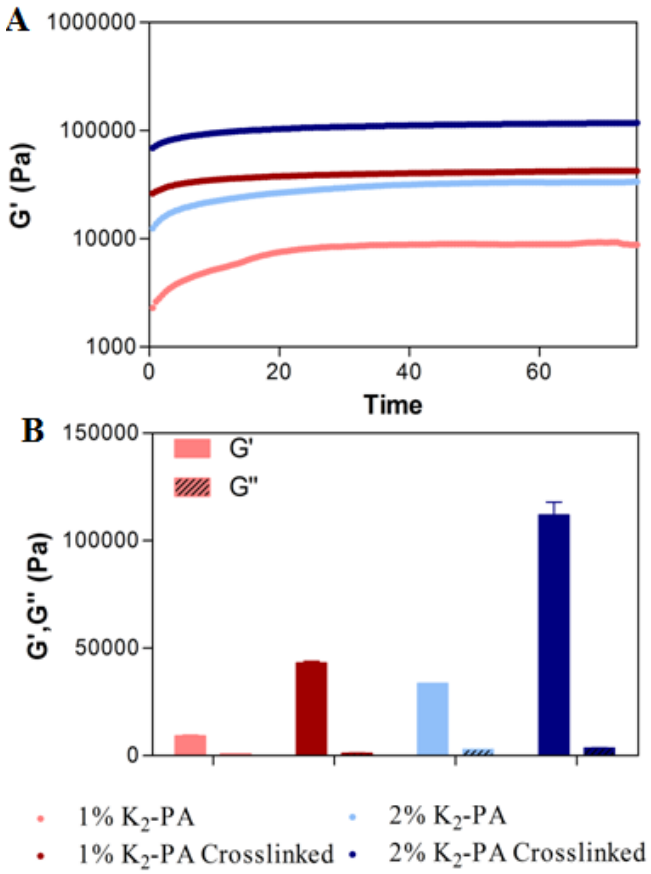

Figure S8. Rheological characterization of the gels. a) Gelation kinetics, b) Equilibrium moduli at pH 10

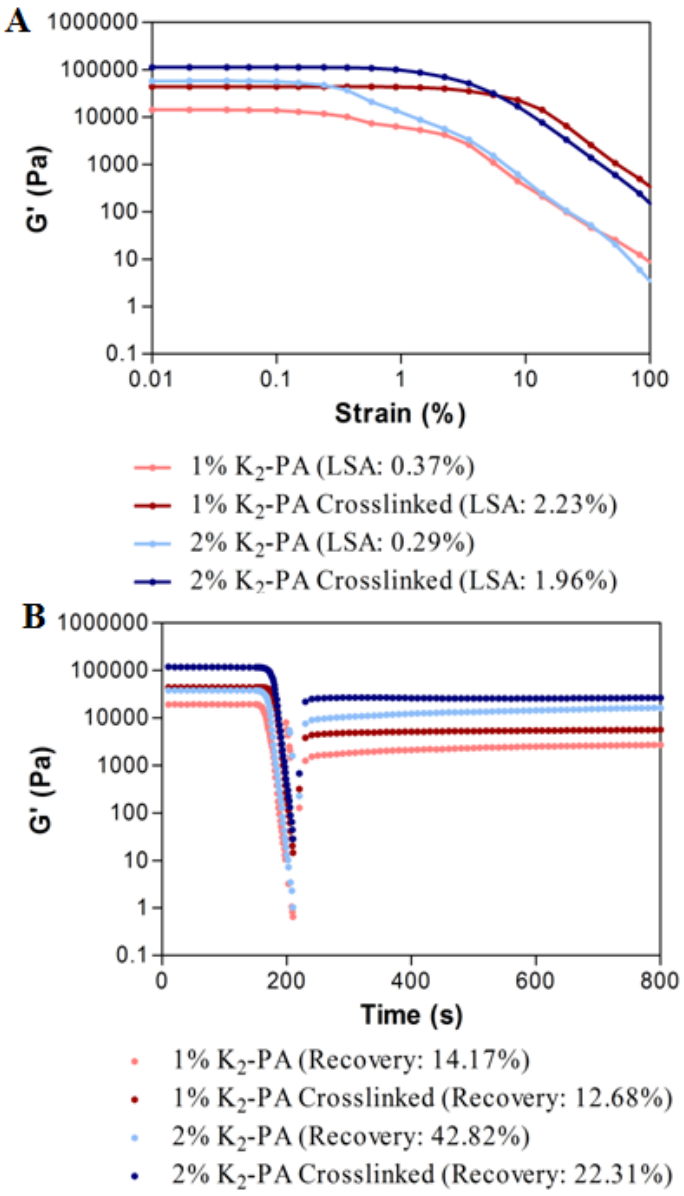

Figure S9. a) Amplitude sweep test and b) self-healing of the hydrogels at pH 10

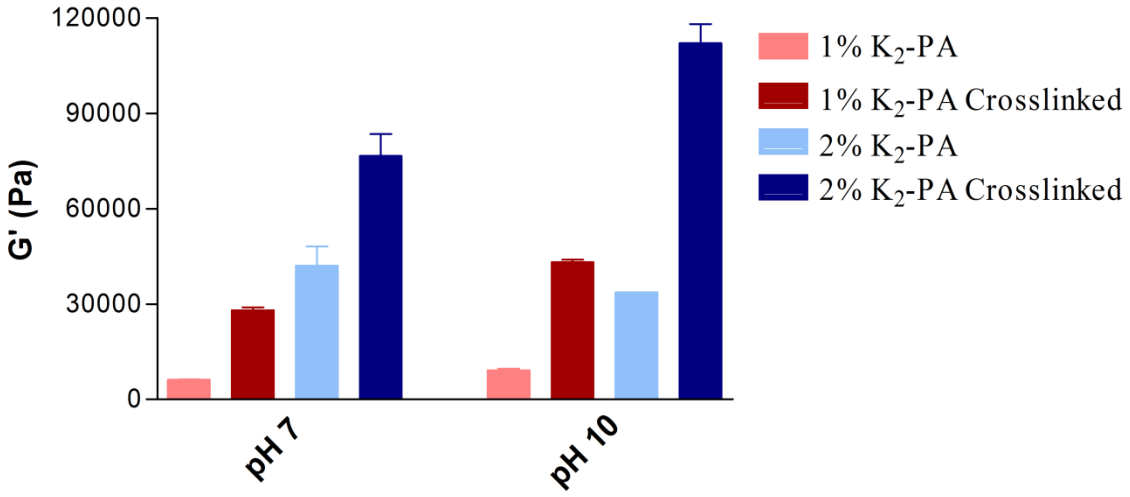

Figure S10. a) Storage moduli of peptide hydrogels

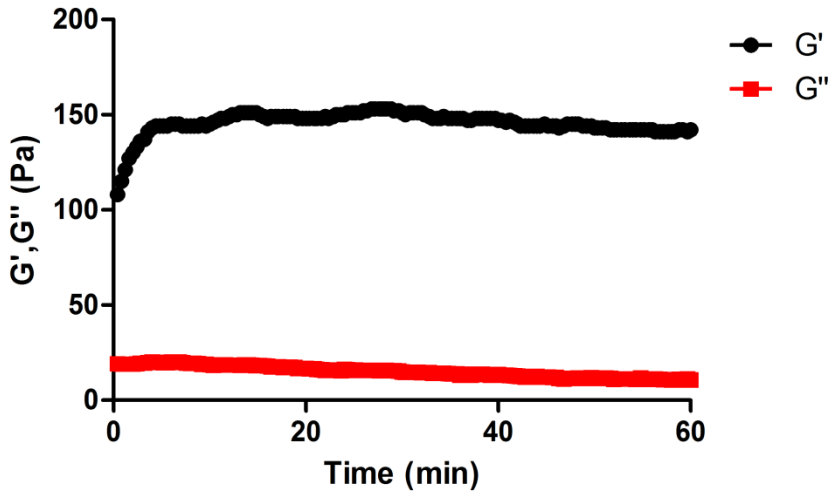

Figure S11.Time sweep analysis of 0.1 wt% ($1.28*10^{-4}$ M) peptide gel