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ESI (Electronic Support Information)

Linear Dependence of Water Proton Transverse Relaxation Rate on Shear Modulus in Hydrogels

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Peptide synthesis and purification

Two peptides (**K11** and **E11**) were synthesized by CEM Liberty microwave peptide synthesizer using standard Solid Phase Peptide Synthesis (SPPS) Fmoc-chemistry. Rink amide MBHA resin and all Fmoc-protected amino acids for SPPS were purchased from aapptec, Inc. The N-terminal of both peptides was acetylated by acetic anhydride. After synthesis, the crude peptides were cleaved by a TFA/TIS/H_2O (95/2.5/2.5 volume ratio) cocktail, precipitated and washed by cold ether. Then the crude peptides were dissolved in water and lyophilized before purification.

Purifications of peptides were conducted on an Agilent 1100 chromatograph system using a ZORBAX 300SB-C18 PrepHT column (21.2 × 250 mm, 7 micron particle size). The flow rate was 5 mL/min. For **K11**, eluent A was H_2O (with 0.1% HCl); eluent B was MeOH (with 0.1% HCl). For **E11**, eluents A was H_2O (with 20 mM NH_4HCO_3, pH 7.0); eluent B was MeOH/H_2O (80/20 v/v, with 20 mM NH_4HCO_3, pH 7.0). Chromatographic method of peptide purification: 0-40% B in 0-60 min, 40-100% B in 60-90 min with linear gradient for each segment.

Fractions of pure peptides were collected and lyophilized to remove solvent and then dialyzed against deionized water to remove excessive salt. The purified peptides were lyophilized and stored in -80 °C before use.

Peptide characterization procedure and results

The purity of each peptide was verified by analytical reverse-phase HPLC. The column was ZORBAX 300SB-C18 (4.6 × 150 mm, 5 micron particle size). The flow rate was 1 mL/min. Eluent A was H_2O (with 0.1% TFA); eluent B was MeOH (with 0.1% TFA). Linear gradient elution (2.5%B/min) was used in analytical HPLC (0-100% B in 40 min). (Figure S1)

All Mass spectrometric analyses of the peptides were carried on a Bruker amaZon Ion Trap mass spectrometer (Figure S2). **K11**, calculated M.W. 1,413 Da, measured 1412.7 Da; **E11**, calculated M.W. 1,419 Da, measured 1418.6 Da (under negative mode).

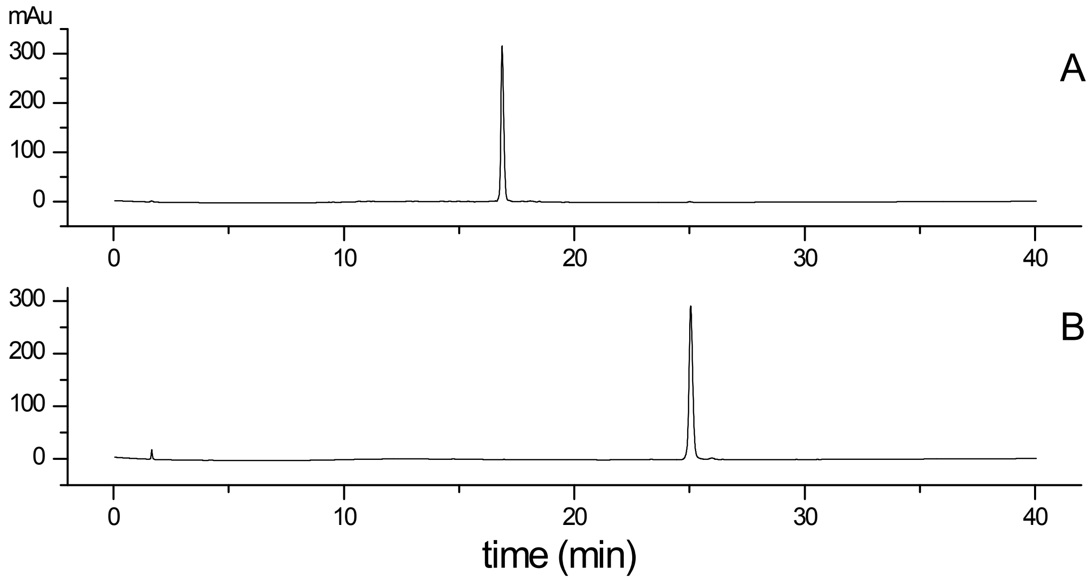

Figure S1. Analytical chromatogram of peptide **K11** (A) and **E11** (B). Eluent A: 0.1% TFA in water; eluent B:0.1% TFA in MeOH. Linear gradient elution method (0-100% B in 40 minutes).

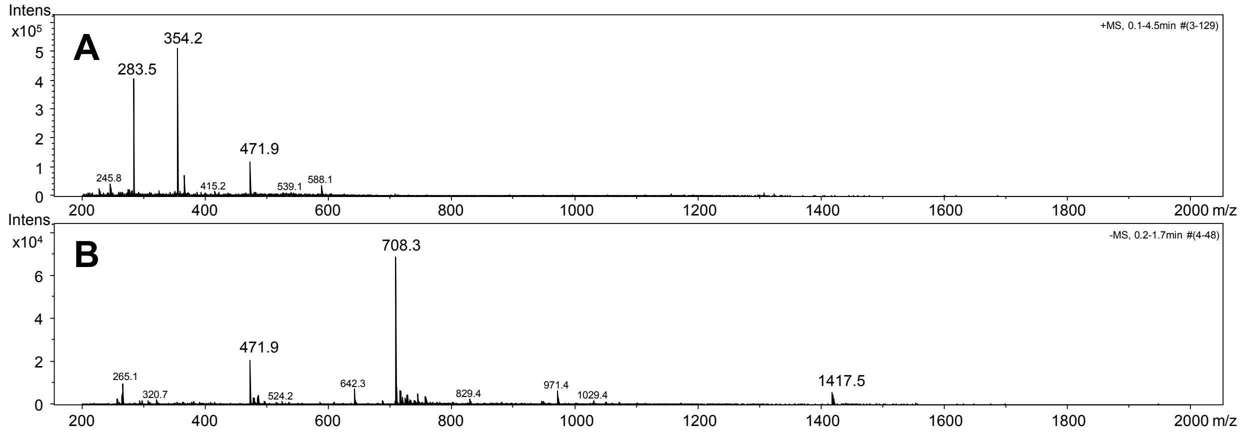

Figure S2. (A) MS of peptide **K11** (calculated M.W. 1,413 Da, positive mode). The 283.5, 354.2 and 471.9 peaks are $(M+H)^+/5$, $(M+H)^+/4$ and $(M+H)^+/3$, respectively. (B). MS of peptide **E11** (calculated M.W. 1,419 Da, negative mode). The 471.9 and 708.3 peaks are $(M-H)^-/3$ and $(M-H)^-/2$, respectively.

Preparation of peptide solutions

K11 and **E11** were each dissolved in 50 mM phosphate buffer of pH 7.4. Afterwards, the pH was adjusted to 7.4 using NaOH. The conductivity of each peptide solution was measured and adjusted by NaCl to 17.0 mS/cm, the same as PBS buffer (50 mM phosphate, 100 mM NaCl, pH 7.4). Conductivity of solutions was measured using a Thermo Scientific Orion 3 star conductivity meter.

After pH and conductivity adjustment, the concentration of each peptide stock solution was measured by UV spectroscopy based the absorption of Trp residues at 280 nm (ε_{280} = 5690 $M^{-1}\cdot cm^{-1}$ per Trp).[1] The measurement was conducted in the presence of *ca*. 6 M GdnHCl to ensure full denaturation. All stock solutions have peptide concentration above 20 mM.

Each stock solution was then diluted by PBS of the same pH (7.4) and conductivity (17 mS/cm) to a final concentration of 4, 8, 10, 12 and 16 mM. Gelation was induced by mixing equal volume of the solutions of **K11** and **E11**. After mixing, the concentration of peptide gelators is: 2 mM + 2mM, 4 mM + 4 mM, 5 mM + 5 mM, 6 mM + 6 mM and 8 mM + 8 mM.

NMR experiments

All NMR experiments were conducted on a Varian 400 MHz spectrometer (9.4 Tesla) at room temperature (22.3 °C), equipped with 5mm broad band probe and Z pulsed-field gradient. The samples were loaded into a 3-mm NMR tube, and insert into a 5-mm NMR outer tube with pre-loaded D_2O and trace amount of TSP. Usually, an NMR sample needs at least 5% deuterium solvent to provide the lock signal, and TSP (or TMS in organic solvent) to provide the chemical shift and intensity reference. But in this way, the D_2O and TSP were isolated from the hydrogel samples, so the samples are exactly the same as those in rheological measurements.

T_1 and T_2 measurements were conducted using the saturation-recovery[2] and CPMG[3] methods, respectively. The self-diffusion coefficients were measured by the PFG (Pulsed-Field-Gradient) NMR method.[4] In all experiments, the relaxation delays were 15 s (larger than $5\times T_1$). In CPMG experiments, the delay between the 180° pulses (2τ) was 120 μs, and to avoid strong water signal overloading the receiver, small angle excitation pulse (about 10 °) was applied. In self-diffusion experiments, the diffusion time was 80 ms. For data presented in Figure 2, gelation proceeded in the NMR tube but outside the NMR spectrometer. For data presented in Figure 3A, gelation proceeded in the NMR tube and inside the NMR spectrometer. There is no noticeable effect of the NMR magnet of gelation.

Rheology experiments

All hydrogels were prepared and measured at 22.3 °C, the same as NMR experiments. The peptide solutions were prepared in the same way as NMR samples. Before mixing, each gelator solution of **K11** or **E11** was centrifuged for 10 min at 8,000 rpm, followed by degassing using house vacuum (1–2 min at 21 Torr). Centrifugation is necessary to remove any suspended particles which might cause the inhomogeneous gelation. Degassing helps to remove excess gases from the solutions, which otherwise might form bubbles during gelation and affect gelation. The two parent peptide solutions, 200 μL of each, were mixed through a Y-shaped connector in the sealed-cell of the rheometer. Rheological measurements started immediately after sample loading.

Dynamic rheological measurements were performed using a NOVA Rheometer (REOLOGICA Instruments, Inc., Sweden) with a null balance system which allows for nano-torque and nano-strain measurement and analysis. The instrument is also equipped with a sealed-cell geometry which prevents dehydration of water-based samples during prolonged measurements. In addition, to exclude possible dehydration of the samples at room temperature, a simple in-house designed system was used to humidify the incoming air used for the sealed-cell bearing. Rheological characterizations of the samples were performed using a 25-mm diameter cone-and-plate steel geometry (4° angle). Time-sweep measurements were conducted at 0.2% strain amplitude and 1 rad/s angular frequency. Shear moduli G of the resulting hydrogels were monitored for 72 h, data points were collected every three minutes until the completion of gelation and G has reached plateau.

References

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Table S1. D, R_1 and R_2 values of water in the parent solutions.

Conc. (mM)	D ($\times 10^{-10}$ $m^2 \cdot s^{-1}$)			R_1 (s^{-1})			R_2 (s^{-1})		
	K11	**E11**	average	**K11**	**E11**	average	**K11**	**E11**	average
2	21.3	20.7	21.0	0.34	0.34	0.34	0.91	0.51	0.71
4	21.0	20.6	20.8	0.35	0.35	0.35	1.42	0.62	1.02
5	20.9	20.6	20.8	0.36	0.36	0.36	1.67	0.71	1.19
6	20.7	20.5	20.6	0.36	0.36	0.36	1.92	0.80	1.36
8	20.5	20.3	20.4	0.36	0.36	0.36	2.38	0.96	1.67