Electronic Supplementary Information (ESI)

Linear Dependency of NMR Relaxation Rates on Shear Modulus in Hydrogels

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Content:

General information

Analytical grade reagents and solvents were purchased from Sigma Aldrich, Inc., Alfa Aesar, Inc., Amresco, Inc., and used without further purification. Rink amide MBHA resin for SPPS was purchased from Chem-impex, Inc. Fmoc-protected amino acids were purchased from Novabiochem, Inc.

Purifications of peptides were conducted on Agilent 1100 HPLC system with a VWD detector. Column: Agilent ZORBAX 300SB-C18 PrepHT (21.2 × 250 mm, 7 micron particle size). Flow rate: 5 mL/min.

Purity of peptides was verified on Agilent 1100 HPLC with a DAD detector. Column: Agilent ZORBAX 300SB-C8 (4.6 × 250mm, 5 micron particle size). Flow rate: 1 mL/min.

Mass spectrometric analyses of the peptides were carried on Finnigan LCQ mass spectrometer.

Materials and Methods

Peptides Synthesis and Purification

Two decapeptides, *formyl*-OF$(OA)_3$OW-*amide* (OAW10) and *formyl*-EF$(EA)_3$EW-*amide* (EAW10), were synthesized on Rink-amide MBHA resin by solid-phase Fmoc-protocol.[1] The *N*-terminal of both peptides were formylated by 2,4,5-trichlorophenyl formate.[2]

The crude peptide were cleaved by a TFA/TIS/H_2O cocktail (trifluoroacetic acid, 95%; triisopropylsilane, 2.5%; water, 2.5%) for 3 hours and the side chain protect group were remove at the same time. Remove most TFA by rotary evaporated, and then the crude peptides were precipitated and washed twice by cold ethyl ether. The precipitation were dissolved in water and lyophilized to give white crude peptide powder.

Crude peptides were purified by preparative reverse-phase HPLC method. For purification of OAW10, solvent A is 0.1% TFA in water and solvent B is 0.1% TFA in acetonitrile; gradient elute, 0-40-100 B% in 0-60-90 min. For EAW10 purification, solvent A is 20 mM NH_4HCO_3 in water (pH=7.0), solvent B is 20 mM NH_4HCO_3 (pH 7.0) in acetonitrile/water (8:2); gradient elute, 0-40-100 B% in 0-60-90 min.

The purity of OAW10 and EAW10 were showed in Figure S1 by reverse-phase HPLC analysis. The solvents used are the same as the preparative HPLC method. Linear gradient (0-100 B% in 40min) were used.

The MS (ESI) results were showed in Figure S2 (OAW10, calculated M.W. 1162.3) and Figure S3 (EAW10, calculated M.W. 1237.2, operate under negative mode).

Peptides were dissolved in PBS (50 mM NaH_2PO_4, 100 mM NaCl, pH 7.0) to form 10.0 mM stock solutions. The concentrations of the stock solutions were determined by UV absorption of the tryptophan residue in each peptide.[3]

The peptide stock solutions were pre-equilibrated prior to experiments either at room temperature (for 25°C gelation) or in cold room (for 5°C gelation). The NMR probe and rheometer geometry were pre-equilibrated at the corresponding temperature as well.

NMR Spectroscopy Measurements

Equal volumes of the peptide stock solutions were mixed and transferred into a 5 mm NMR tube. All samples contained 10% D_2O and about 17 μM TSP (trimethylsilyl-

2,2,3,3-tetradeutero propionic acid) as a chemical shift reference at 0.0 ppm in the ^{1}H spectra.

All NMR experiments were carried on a Varian INOVA 500 spectrometer. NMR data acquisition started about 10 min after mixing the two peptide solutions. Proton signal intensity, diffusion coefficient D, relaxation times T_1 and T_2, were measured in a sequential manner and repeated every hour, until no noticeable further decrease in ^{1}H signal intensity.

Pre-saturated water suppression was applied in all experiments. Diffusion coefficients were measured by the BPP-LED pulse sequence.[4] The inversion-recovery[5] and CPMG[6] pulse sequences were used to measure the spin-lattice and spin-spin relaxation times T_1 and T_2, respectively.

After the ^{1}H NMR signals reached plateau, a temperature switch experiment was conducted. The gel initially formed at 25°C was cooled to 5°C and kept at 5°C for 4 hr for NMR measurements. The gel initially formed at 5°C was heated to 25°C and kept at 25°C for additional NMR measurements until a new plateau was reached.

Dynamic Rheometry Measurements

200 µL of each peptide solution were mixed directly in the 25 mm diameter cone-and-plate steel geometry through a Y-shaped connector to form the hydrogel in-situ.

Dynamic rheological measurements were performed using a NOVA Rheometer (REOLOGICA Instruments, Inc., Sweden) with a sealed-cell geometry and a simple in-house built humidifier which prevents dehydration of the water-based samples during prolonged measurements (Figure S4). Time-sweep measurements were conducted at 0.2% strain amplitude and 1 rad/s angular frequency. Frequency-sweep measurements were conducted at the respective temperatures with 0.2% strain amplitude, while the frequency was varied from 0.01 to 100 rad/s with 18 data points per frequency decade (Figure S5). All gels demonstrate very similar G' and G'' vs. angular frequency profiles. Such frequency profiles are characteristic for viscoelastic solid-like materials, with $G''(\omega)$ showing some signs of relaxation at higher frequencies around 100 rad/sec (especially at 5°C, Figure S5). The G' has very slight dependence on the angular frequency within the studied range from 0.01 to 100 rad/sec, confirming the formation of stable solid-like hydrogel network with little or no mobility at time scales up to $t = 2\pi/\omega \sim 600$ s, *i.e.*, up to the longest measurement duration. After the frequency-sweep measurements, a time-sweep of

3 hr was performed on the gel at 0.2% strain amplitude, 1 rad/s frequency to confirm that the gel remains undisturbed by the frequency-sweep (Figure S6). Strain-sweep measurements were then performed with a single integration cycle at 1 rad/s angular frequency, within the range of strain amplitudes from 0.1% to 100% with 23 data point per decade. The gel formed at 25°C is much stronger than the gel formed at 5°C in terms of γ_{yield} (1.5% vs. 0.5%). Heating up for the 5°C $\rightarrow$ 25°C gel results in the insignificant increase in γ_{yield} from 0.5% to 0.6%, while cooling down for the 25°C $\rightarrow$ 5°C gel makes the gel more brittle (γ_{yield} drops form 1.5% to 0.8%) despite the fact that this gel has higher shear modulus (Figure S7).

Temperature switch was performed similar to the NMR studies described above. The gel formed at 25°C was cooled down to 5°C, and the gel formed at 5°C was heated up to 25°C (linear temperature gradient was 0.4°C/min for both cooling and heating). The changes in the viscoelastic properties of the materials were monitored by time-sweep, frequency-sweep and strain-sweep experiments with the same parameter settings as above.

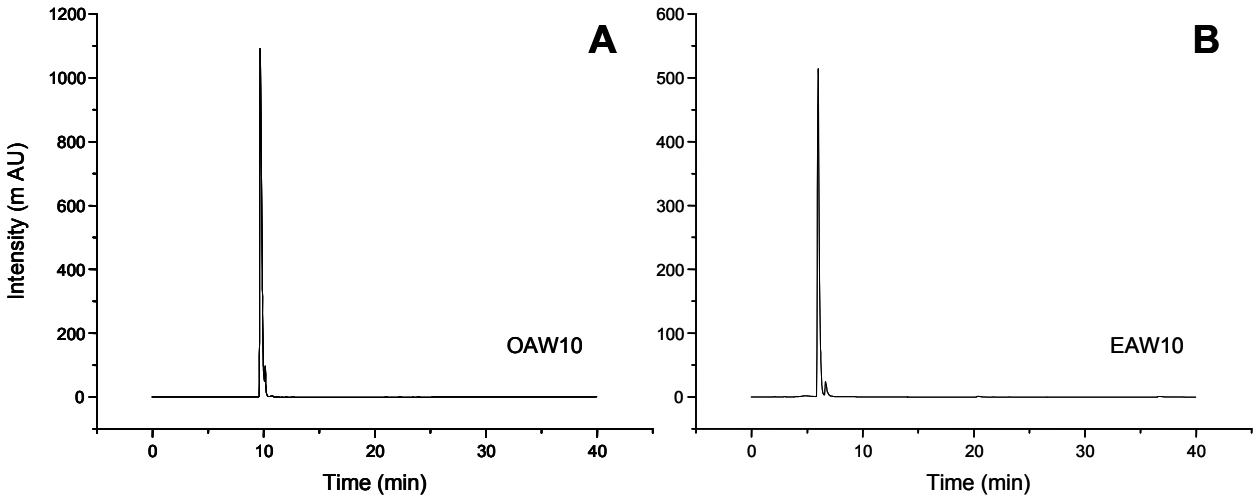

Figure S1. The reverse-phase HPLC chromatogram of peptide OAW10 (A) and EAW10 (B). (A), solvent A is 0.1% TFA in water and solvent B is 0.1% TFA in acetonitrile; (B), solvent A is 20 mM NH_4HCO_3 in water (pH=7.0), solvent B is 20 mM NH_4HCO_3 (pH 7.0) in acetonitrile/water (8:2). Gradient elute method was used (0-100 B% in 40 min).

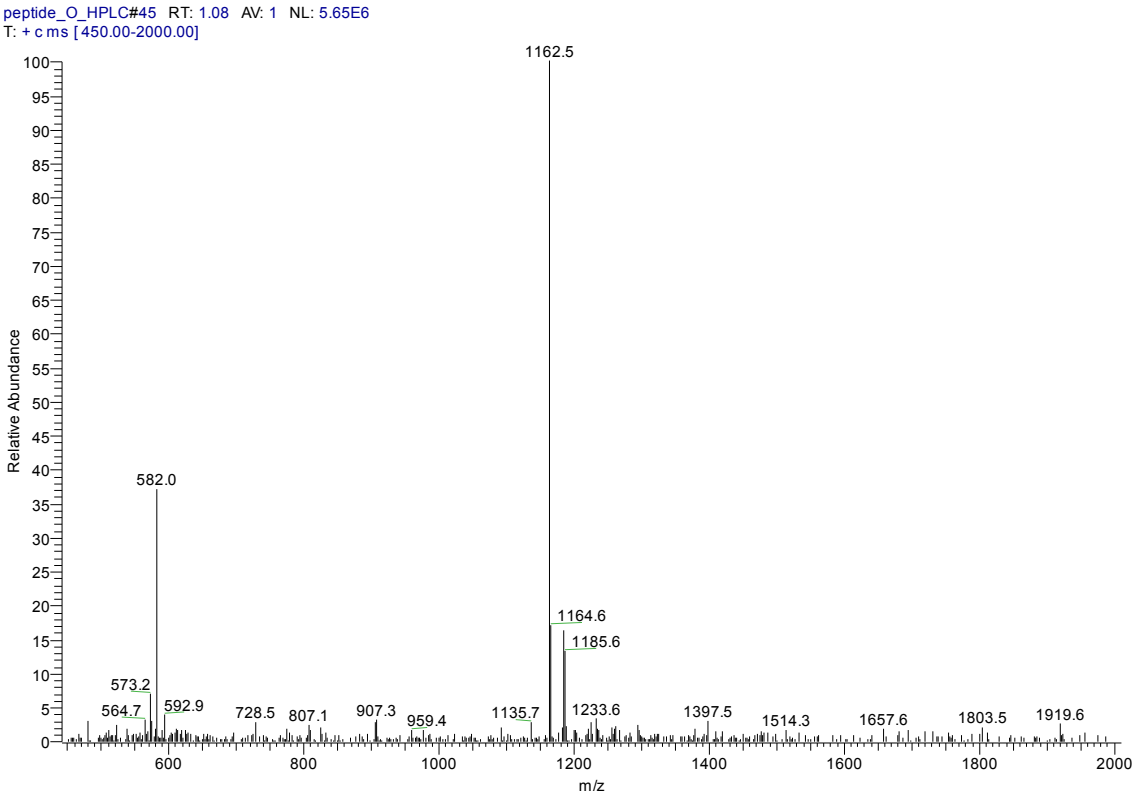

Figure S2. MS (ESI) of OAW10 (calculate M.W. is 1162).

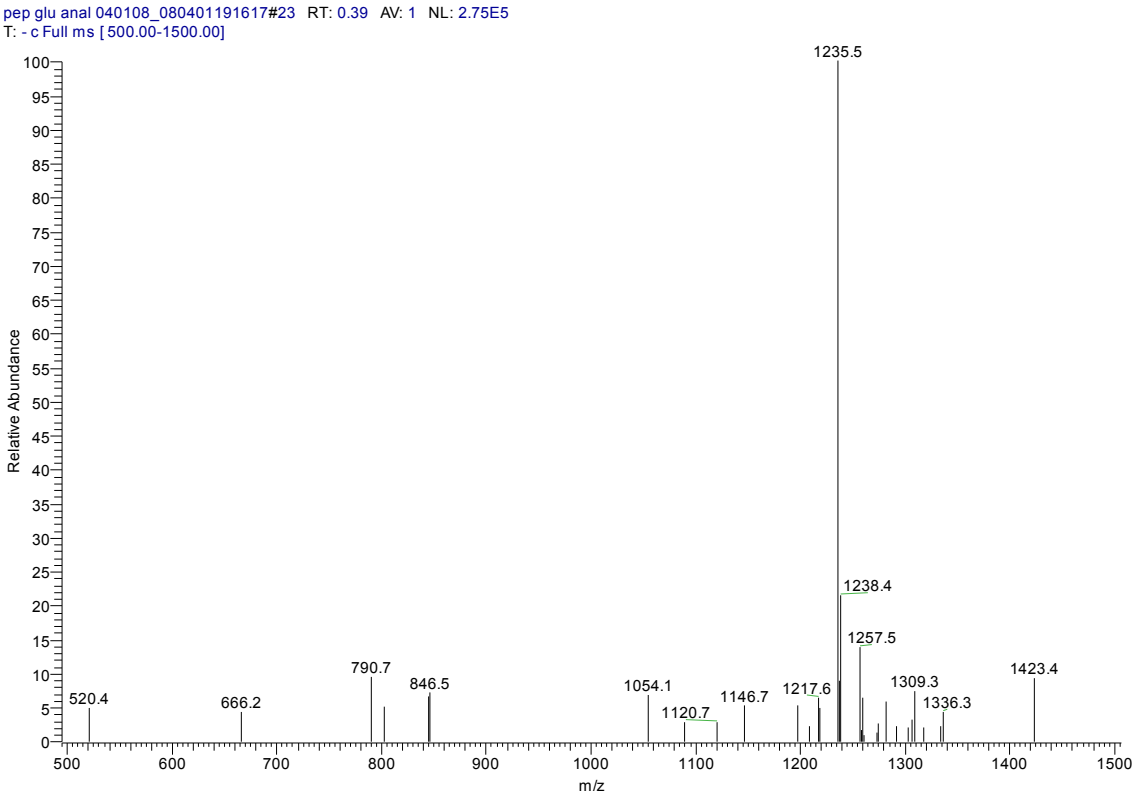

Figure S3. MS (ESI) of EAW10 (calculate M.W. is 1237). Experiment carried on negative mode.

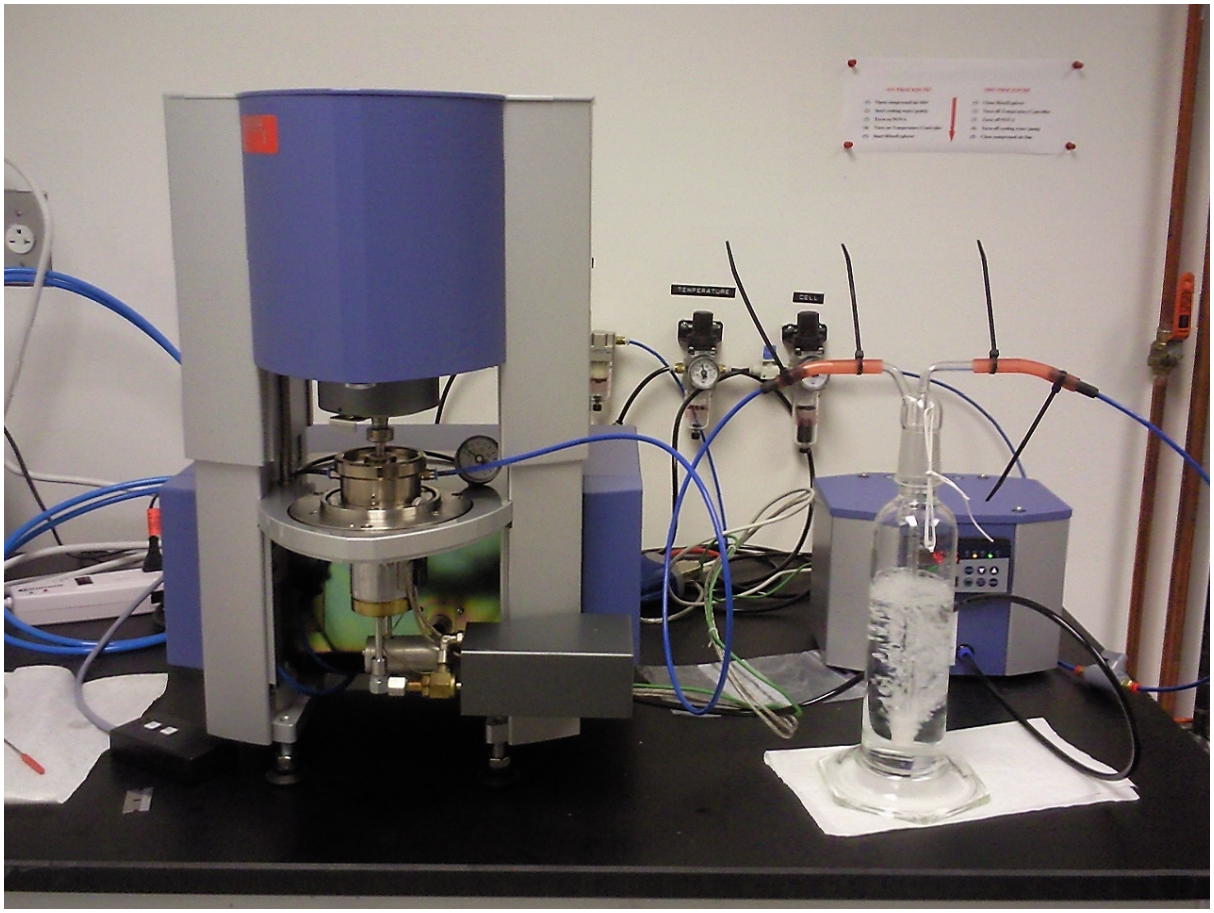

Figure S4. Sealed-cell NOVA Rheometer with the in-house designed simple humidifier used to prolong measurements at 25 °C.

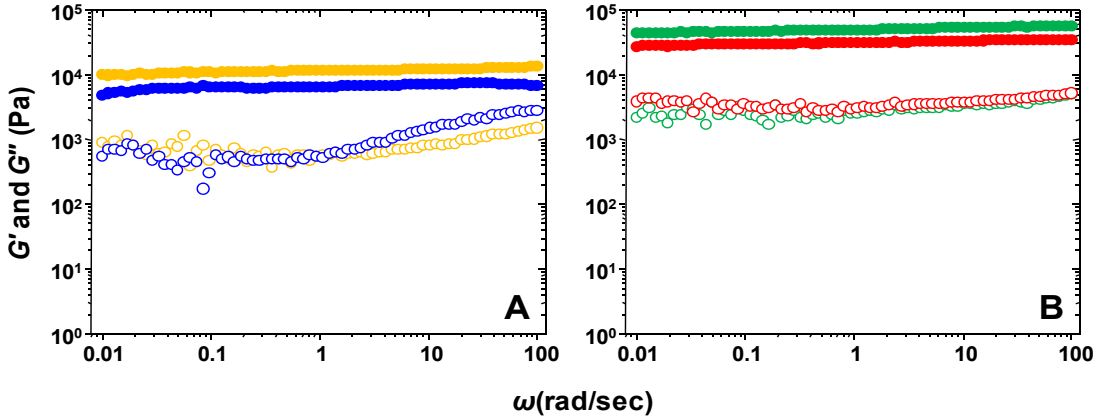

Fig. S5. Frequency-sweep measurements of the hydrogels. *G′* (solid circles), *G″* (open circles). (A) Frequency-sweep of the 5°C gel (blue) and the 5°C → 25°C gel (orange). (B) Frequency-sweep of the 25°C gel (red) and the 25°C → 5°C gel (green).

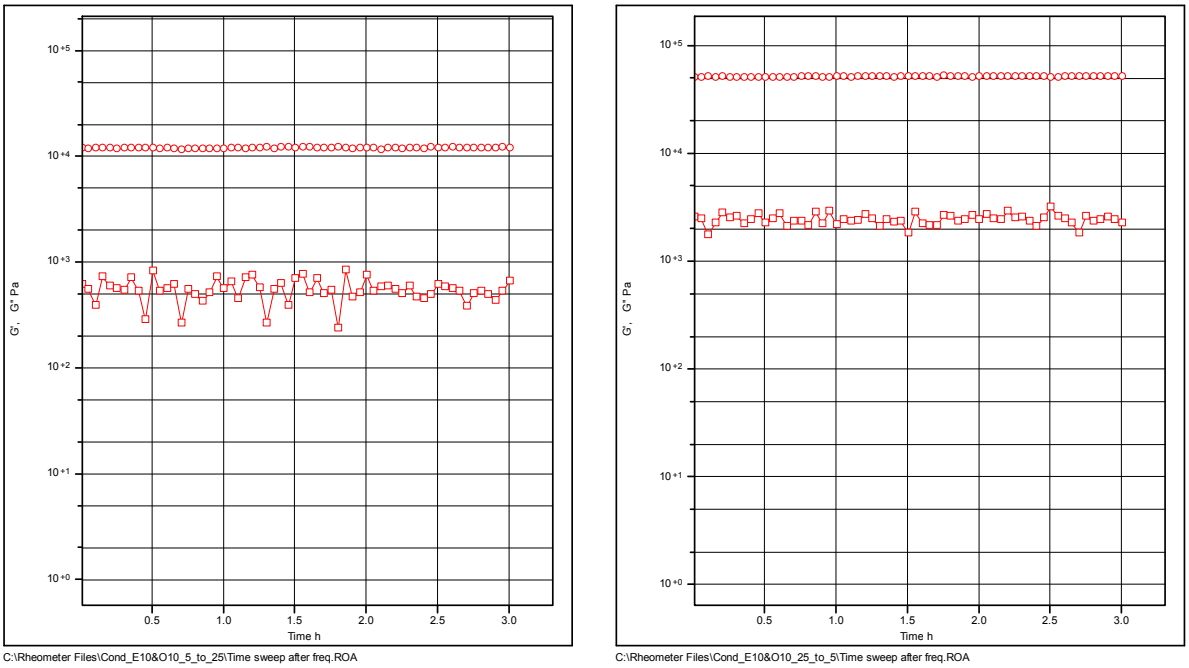

Figure S6. Time-sweep measurements of viscoelastic properties of decapeptide hydrogels performed after frequency-sweeps confirming the stability of the gels. Left: the 5°C → 25°C gel. Right: the 25 °C → 5 °C gel.

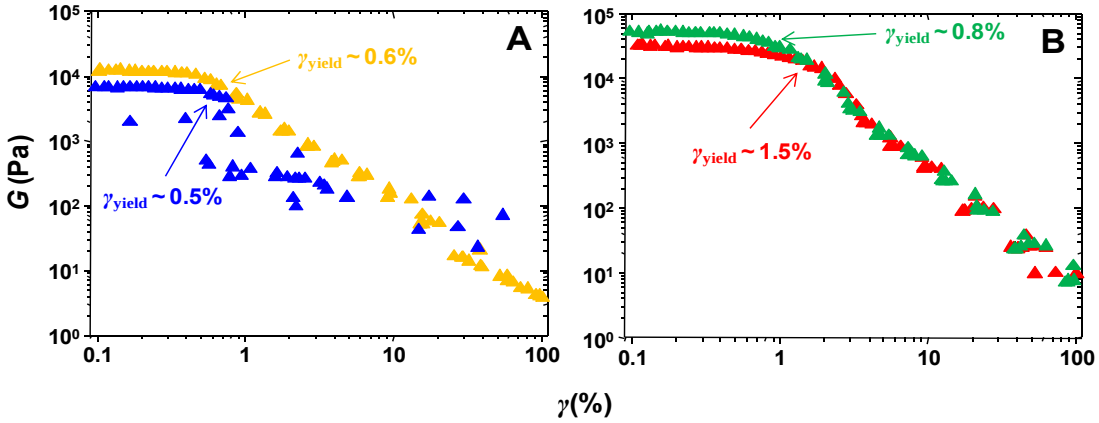

Fig. S7. Shear modulus G *vs.* strain γ. $G = (G' + G'')^{½}$. (A) Strain-sweep of the 5°C gel (blue) and the 5°C → 25°C gel (orange) (B) Strain-sweep of the 25°C gel (red) and the 25°C → 5°C gel (green). The yield point is indicated by an arrow for each gel.

Table S1. Temperature effects on the viscoelastic properties of hydrogels

	G' (kPa)	G'' (kPa)	γ (%)
5°C	5.0	0.2	0.5
25°C	35.0	1.5	1.5
5°C $\rightarrow$ 25°C	11.0	0.6	0.6
25°C $\rightarrow$ 5°C	50.0	2.0	0.8

G' and G'' are storage and loss moduli, respectively; γ is yield strain value.

Table S2. Intercepts (a_i), slopes (b_i) and coefficients of determination (R^2) for fitting Eqn. 5.

	R_1 vs. G			R_2 vs. G		
	a_1 [s^{-1}]	b_1 [$10^{-6}Pa^{-1}\cdot s^{-1}$]	R^2	a_2 [s^{-1}]	b_2 [$10^{-6}Pa^{-1}\cdot s^{-1}$]	R^2
-CH_3 of peptides	3.833	83	0.996	5.463	547	0.994
-CH_2- of peptides	3.658	72	0.990	6.571	400	0.991
-CH_3 of TSP	1.851	39	0.996	3.112	114	0.981
	R_1 vs. G'			R_2 vs. G'		
	a_1 [s^{-1}]	b_1 [$10^{-6}Pa^{-1}\cdot s^{-1}$]	R^2	a_2 [s^{-1}]	b_2 [$10^{-6}Pa^{-1}\cdot s^{-1}$]	R^2
-CH_3 of peptides	3.833	83	0.996	5.465	547	0.994
-CH_2- of peptides	3.658	72	0.990	6.573	400	0.991
-CH_3 of TSP	1.851	39	0.996	3.113	114	0.981
	R_1 vs. G''			R_2 vs. G''		
	a_1 [s^{-1}]	b_1 [$10^{-6}Pa^{-1}\cdot s^{-1}$]	R^2	a_2 [s^{-1}]	b_2 [$10^{-6}Pa^{-1}\cdot s^{-1}$]	R^2
-CH_3 of peptides	3.648	2,127	0.998	4.431	13,817	0.972
-CH_2- of peptides	3.508	1,834	0.982	5.793	10,139	0.974
-CH_3 of TSP	1.766	988	0.997	2.837	2,944	0.997

References

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