Electronic Supplementary Information (ESI)

The Effect of Ionic Strength on the Mechanical, Structural and Transport Properties of Peptide Hydrogels

Yue Feng,[a] Marc Taraban,[b] Yihua Bruce Yu* [a,b]

[a]*Department of Pharmaceutical Sciences, School of Pharmacy, University of Maryland, Baltimore, MD 21201, USA*

[b]*Fischell Department of Bioengineering, University of Maryland, College Park, MD 20742, USA*

*Corresponding author: byu@rx.umaryland.edu

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 and Fmoc-protected amino acids for SPPS were purchased from aapptec, Inc.

Purifications of peptides were conducted using an Agilent 1100 preparative 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. Temperature: room temperature.

Purity of peptides was verified on an Agilent 1100 analytical HPLC with a DAD detector. Column: Agilent Eclipse XDB-C18 (4.6 × 150mm, 5 micron particle size). Flow rate: 1 mL/min. Temperature: room temperature.

Mass spectrometric analyses of the peptides were carried using a Bruker AmaZon X ion trap mass spectrometer.

Peptides purification

Crude peptides were purified by preparative reverse-phase HPLC method. For the purification of K11, eluent A is 0.1% HCl in water and eluent B is 0.1% HCl in methanol; gradient elution, 0-40-100 B% in 0-60-90 min. For E11 purification, eluent A is 20 mM NH_4HCO_3 in water (pH=7.0), eluent B is 20 mM NH_4HCO_3 (pH 7.0) in methanol/water (8:2); gradient elution, 0-40-100 B% in 0-60-90 min.

The purity of E11 and K11 is shown in Figure S1 by reverse-phase HPLC analysis. The eluents used are the same as the preparative HPLC method. Gradient elution was used (for K11, 0-40 B% in 16 min; for E11, 0-30 B% in 12 min).

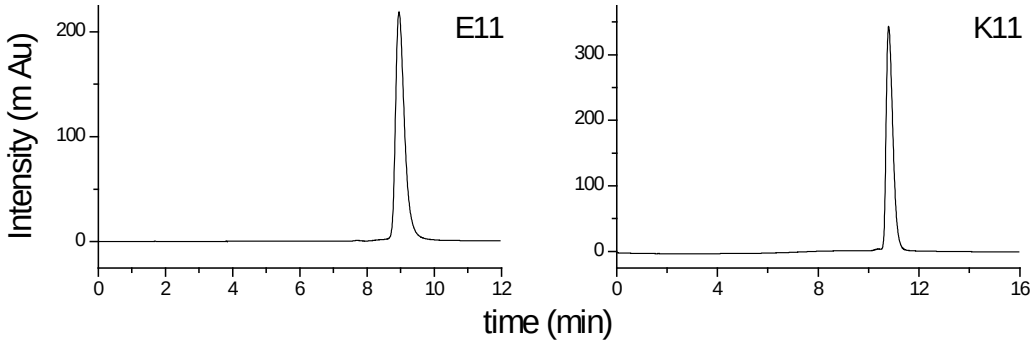

Figure S1. Reverse-phase HPLC chromatograms of peptides E11 and K11. For E11, eluent A is 20 mM NH_4HCO_3 in water (pH=7.0), eluent B is 20 mM NH_4HCO_3 (pH 7.0) in methanol/water (8:2), gradient elution method was used (0-30 B% in 12 min). For K11, eluent A is 0.1% TFA in water and eluent B is 0.1% TFA in acetonitrile, gradient elute method was used (0-40 B% in 16 min). The experiments were conduct at room temperature.

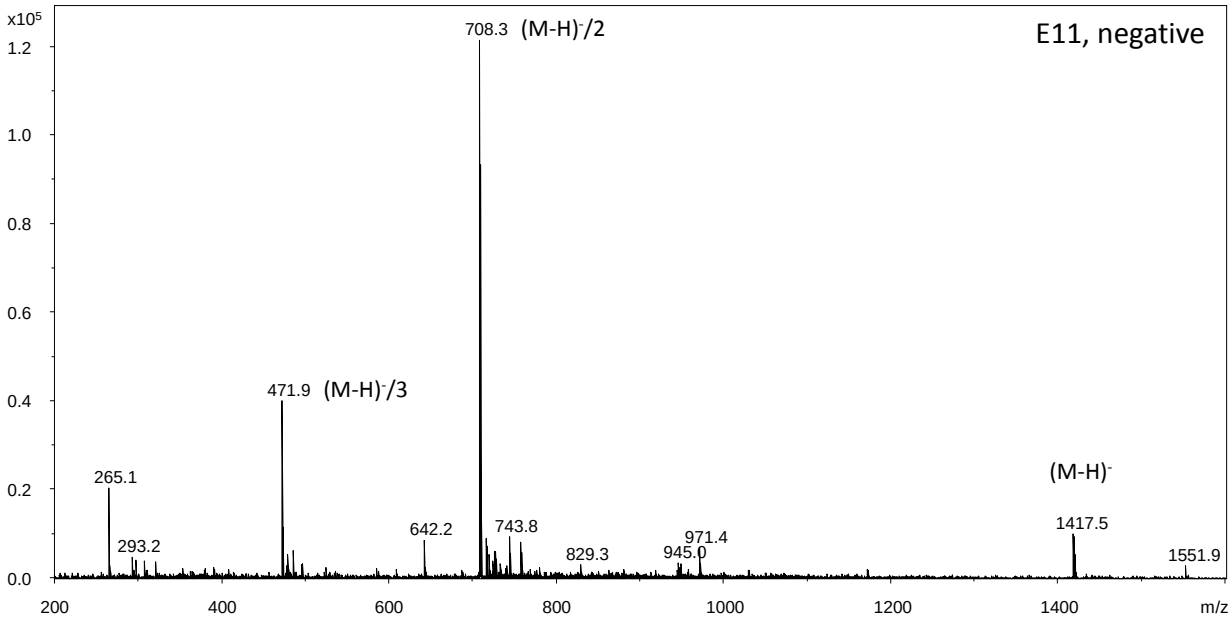

Figure S2. MS of peptide E11 (calculated M.W. 1,419 Da, negative mode).

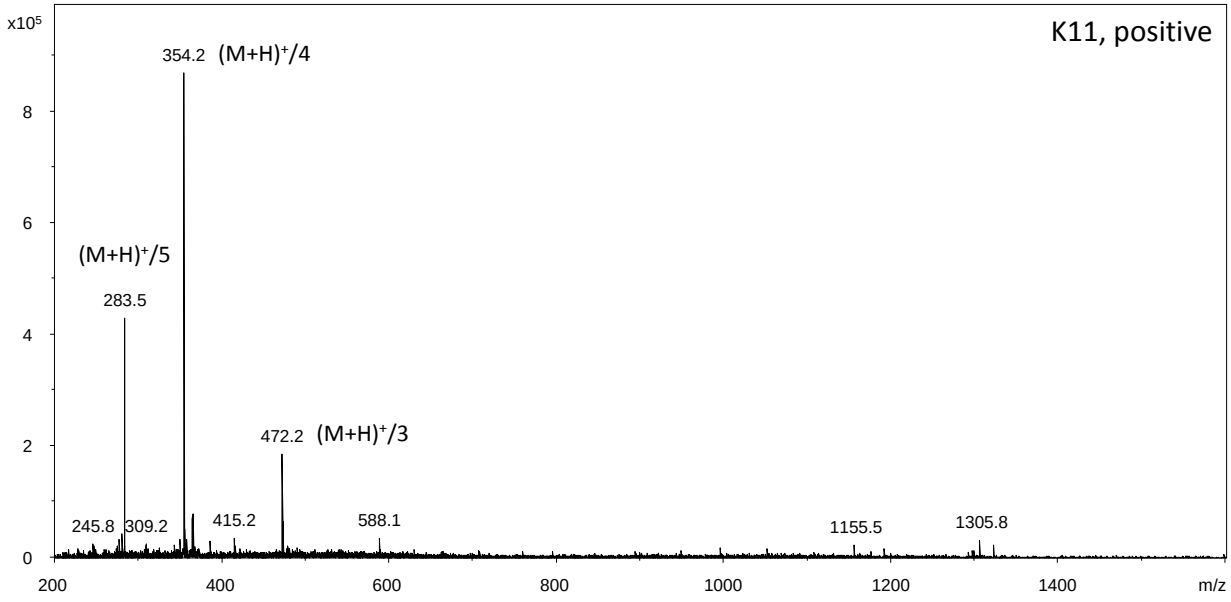

Figure S3. MS of peptide K11 (calculated M.W. 1,413 Da).

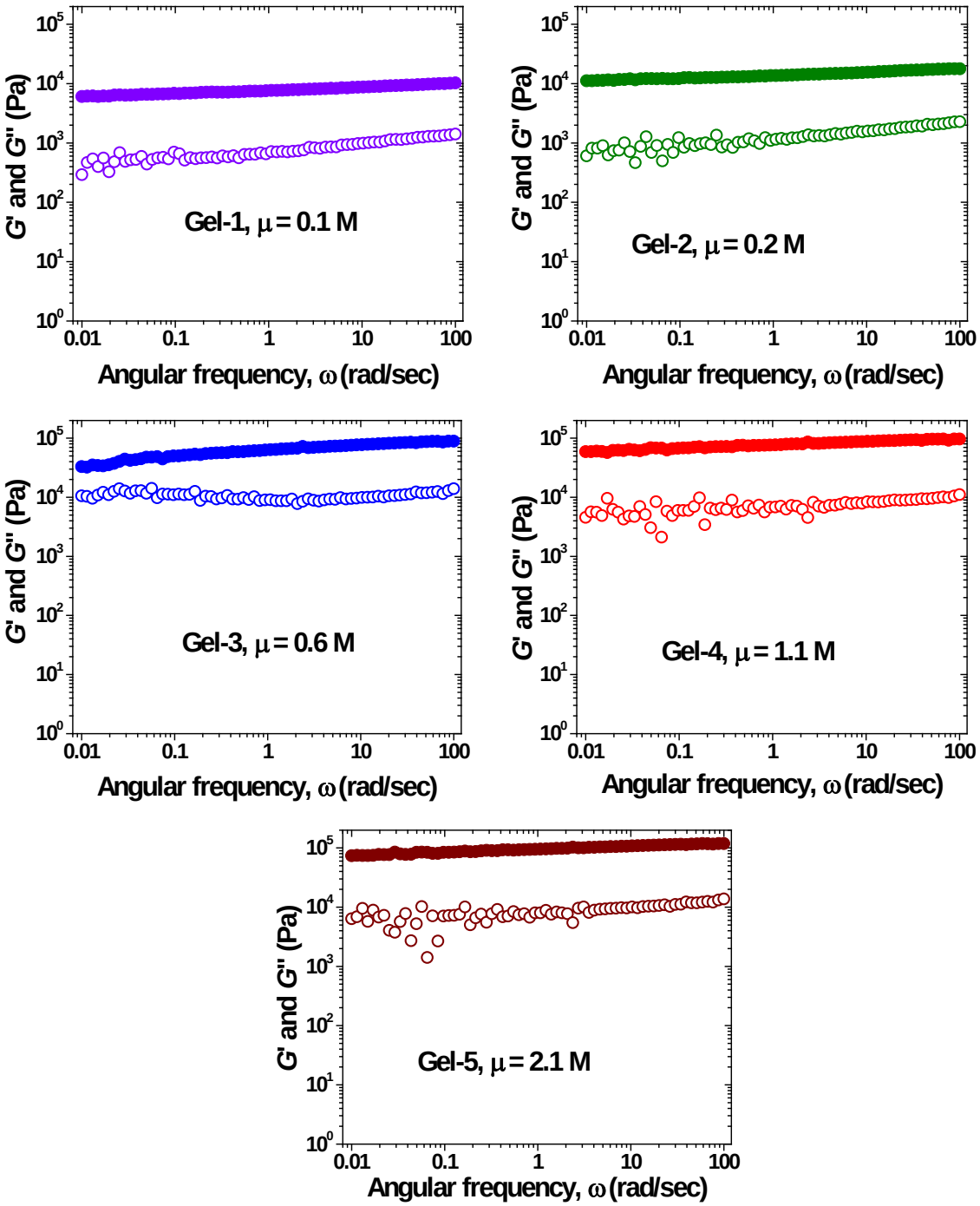

Figure S4. Frequency-sweep data for hydrogels **Gel-1 – Gel-5** after 72 h of gelation. Violet: **Gel-1** (μ = 0.1 M), green: **Gel-2** (μ = 0.2 M), blue: **Gel-3** (μ = 0.6 M), red: **Gel-4** (μ = 1.1 M), brown: **Gel-5** (μ = 2.1 M).

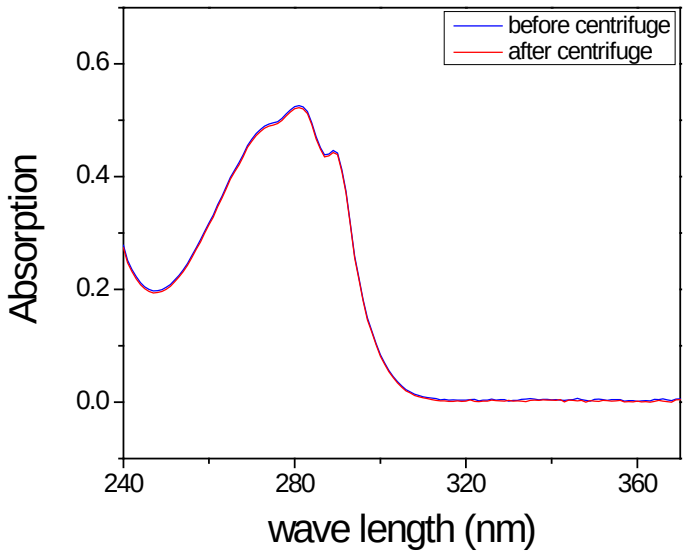

Figure S5. UV spectra of peptide stock solution A-5, before and after centrifugation (8000 rpm, 10 min.). The results show that after centrifugation, the concentration of peptide does not change, suggesting that at high ionic strength (2.1 M), there is no self-assembly of the peptide.

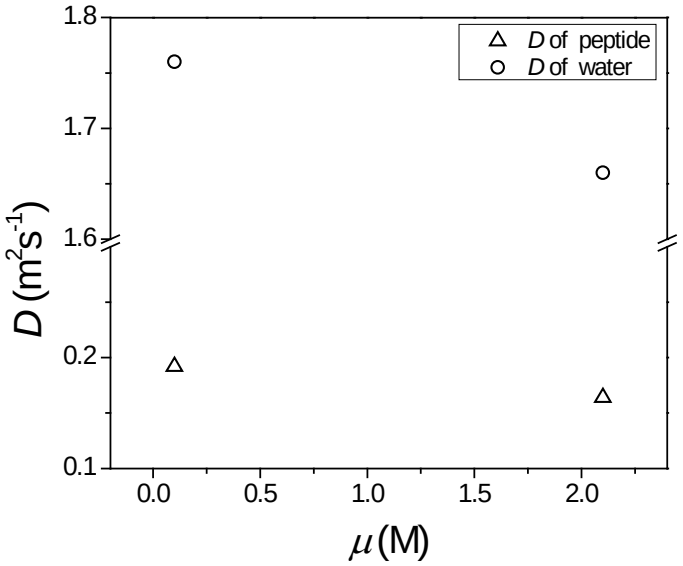

Figure S6. Diffusion coefficients (*D*) of peptide and H_2O in A-1 (μ=0.1) and A-5 (μ=2.1) stock solutions. The *D*(peptide)/*D*(H2O) ratio in A-1 and A-5 are very close (0.11 and 0.10, respectively). This suggests that the peptide does not aggregate at high ionic strength.

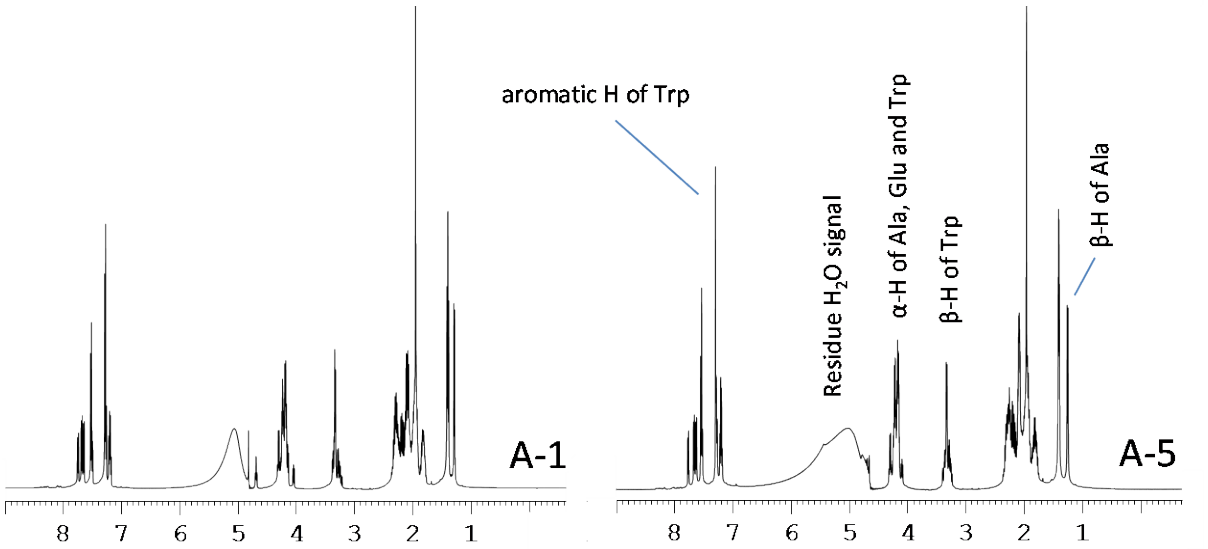

Figure S7. ^{1}H NMR spectra of stock solutions A-1 and A-5. The sharp peaks indicate that the peptide self-assembly did not happen in both lower and higher ionic strength solutions.

Table S1. Changes of the radii of gyration of the cross-section R_c and the maximum cross-sectional dimensions d_{max} in hydrogel fibers formed at different ionic strength over time.[a]

Time (hrs)	**Gel-1** (0.1 M)		**Gel-2** (0.2 M)		**Gel-3** (0.6 M)		**Gel-4** (1.1 M)		**Gel-5** (2.1 M)	
	R_c, Å	d_{max}, Å	R_c, Å	d_{max}, Å	R_c, Å	d_{max}, Å	R_c, Å	d_{max}, Å	R_c, Å	d_{max}, Å
0.5	12.3	45	7.8	28	6.6	20	6.7	20	9.2	29
1.5	14.8	50	11.6	46	7.0	23	6.8	21	9.8	29
3	14.9	50	14.9	55	7.5	23	7.0	23	10.1	32
5	15.2	53	14.9	55	7.9	26	7.7	24	10.3	32
7	15.6	56	15.4	56	8.8	26	8.1	26	10.9	35
24	16.6	62	16.0	59	14.4	53	12.7	44	13.7	47
48	18.0	68	17.0	62	15.6	56	14.2	50	14.3	50
72	20.1	77	19.1	72	16.1	62	14.7	51	14.3	51

[a] Corresponding values of ionic strength for each gel are given in the parentheses.