

## SUPPLEMENTARY INFORMATION

### Supramolecular Self-Assembly to Control Structural and Biological Properties of Multicomponent Hydrogels

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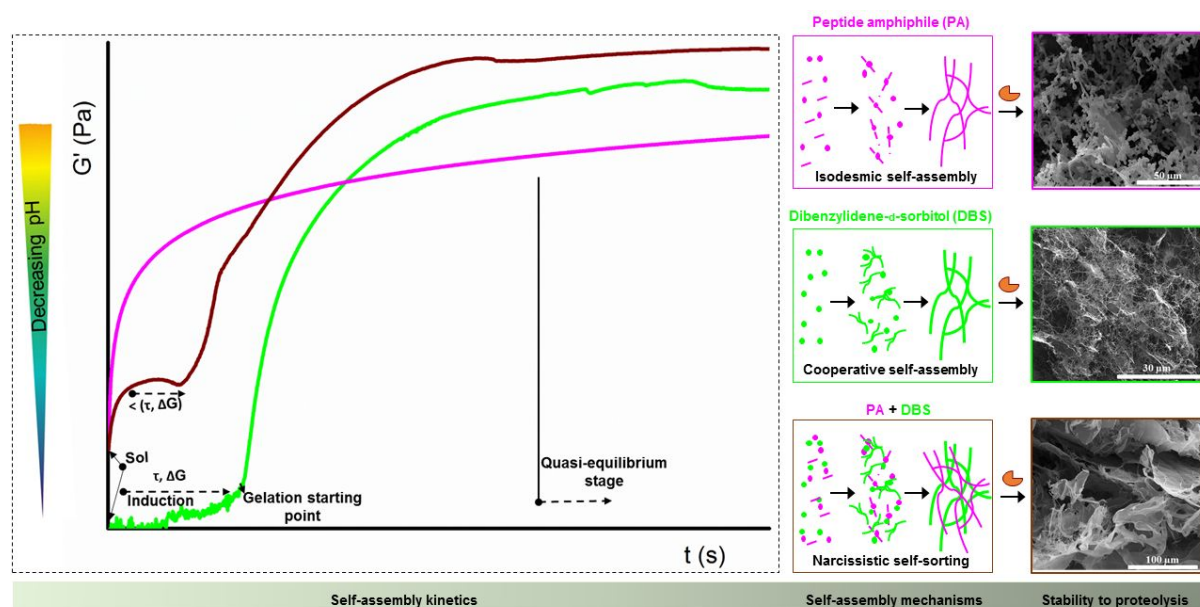
#### Contents

1. Synthesis
2. Overview of multicomponent self-assembly and benefits
3. ThT fluorescent microscopy assay
4. CD spectra for ThT interaction
5. TEM characterization of nanostructures of **PA-E3**, **DBS-COOH** and mixtures
6. Small-Angle Neutron Scattering (SANS) measurements
7. Rheological kinetics for **PA-E3**, **DBS-COOH** and **PA-E3/DBS-COOH** mixture
8. Avrami Equations
9. Molecular dynamics computation
10. Amplitude sweeps, critical strain and self-recovery of hydrogels
11. UV-TIC spectra for hydrogel digestion
12. References

## 1. Synthesis

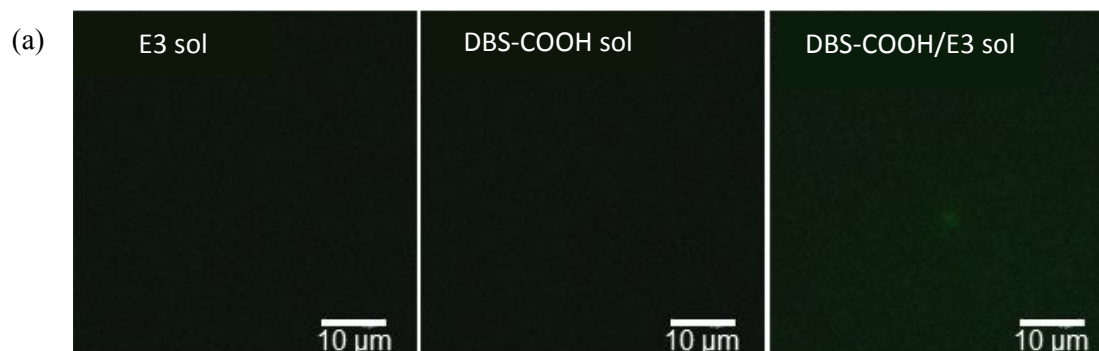
PA-E3 and DBS-COOH were synthesized and characterized as previously reported elsewhere.<sup>1,2</sup>

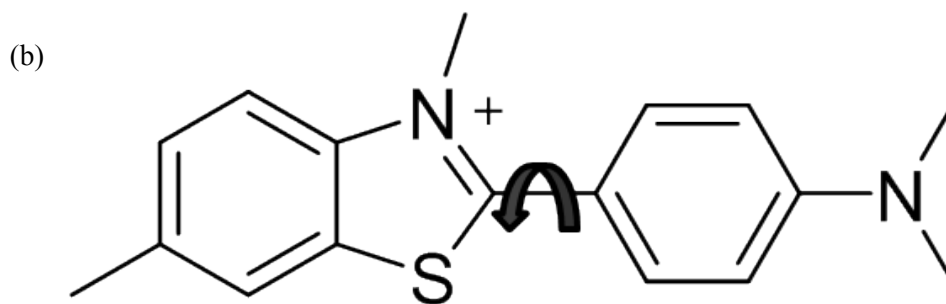
## 2. Overview of multicomponent self-assembly and benefits



**Figure S1.** Schematic representation of self-assembly kinetics, self-assembly mechanisms and response to proteolysis by peptide amphiphile (PA-E3), DBS-COOH and PA-E3/DBS-COOH.

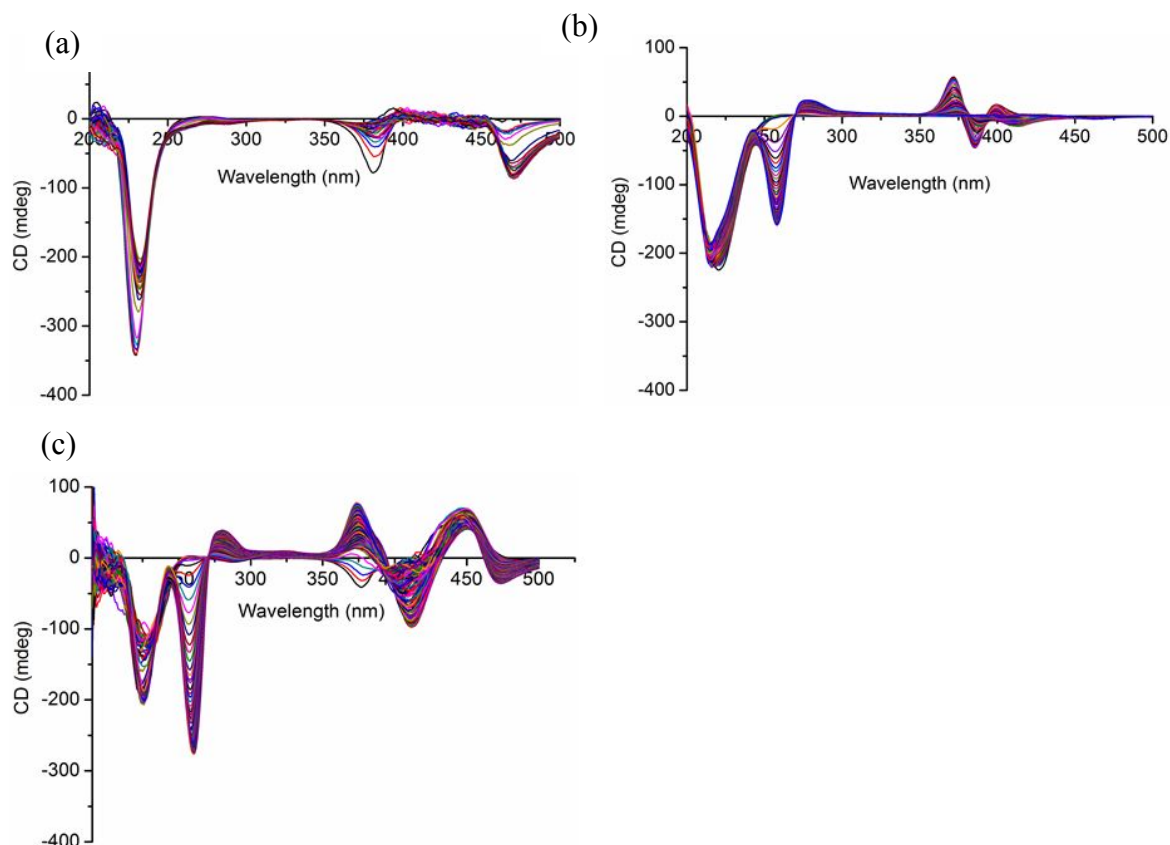
## 3. Thioflavin T (Th T) fluorescence assay





**Figure S2.** LSCM images of **PA-E3**, **DBS-COOH** and **DBS-COOH/PA-E3** sols showing that ThT showed a weak fluorescent signal in the sol state of the gelators. (b) Molecular structure of ThT showing the torsion angle between the benzothiazole and aminobenzene.

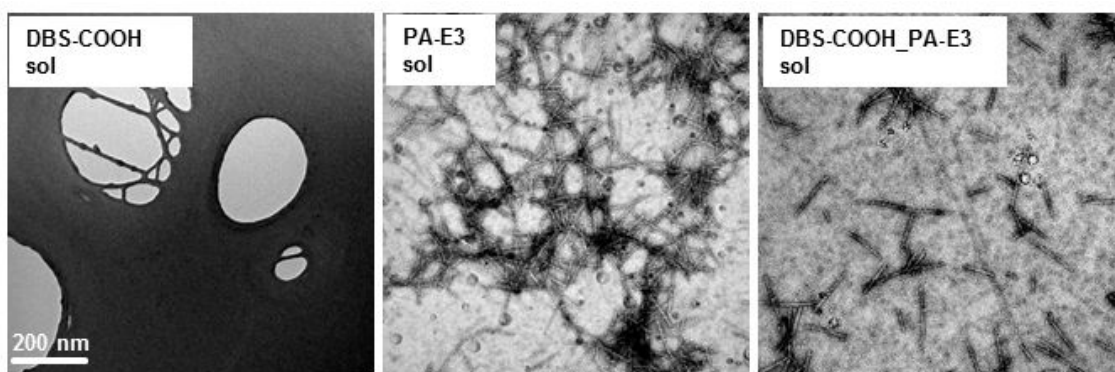
#### 4. CD measurement for ThT interactions



**Figure S3.** Circular dichroism spectra confirming that ThT was incorporated into nanofibers of (a) **PA-E3**, (b) **DBS-COOH** and (c) their equimolar in a differential and time-dependent manner. The band at 218 nm is a circular dichroic signature of gluconic acid which results from the base-catalysed hydrolysis of GdL. It is also expected that **PA-E3** should display the

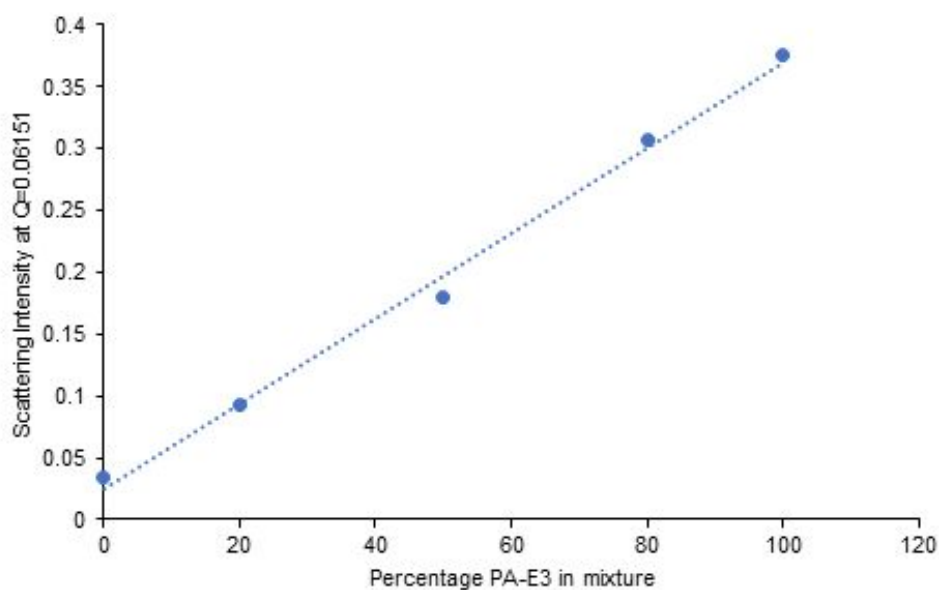
characteristic  $\beta$ -sheet signature of peptide amphiphiles at this region. In (b), DBS-COOH exhibits a strong negative absorption at 254 nm (due to the chiral sugar backbone) and a weak positive signal at 280 nm (due to the aromatic chromophores). ThT exhibit different CD signatures depending on the chiral environment.

## 5. TEM characterization of nanostructures of PA-E3, DBS-COOH and their co-assemblies



**Figure S4.** TEM images showing the pre-gelation nanostructures of **PA-E3** alone sol and in **PA-E3/DBS-COOH** admixtures against **DBS-COOH** alone sol.

## 6. Small-Angle Neutron Scattering (SANS) analysis of hydrogel nanostructures

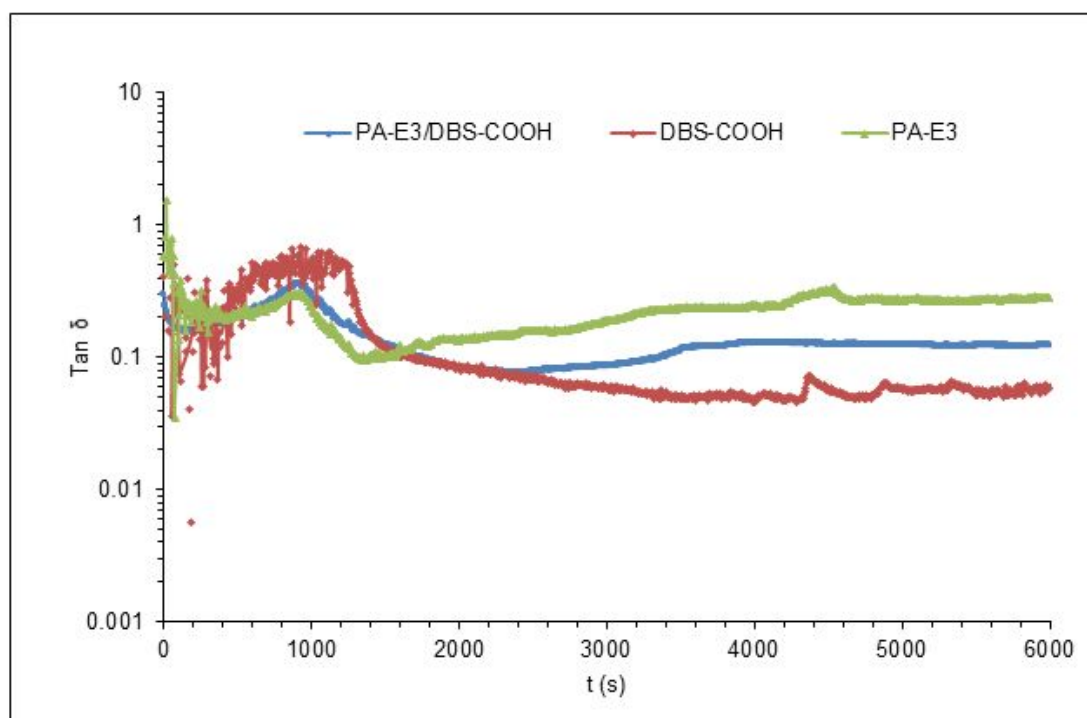


**Figure S5.** A plot of intensity at Q of 0.06151 against % **PA-E3** in the mixture.

**Table S1.** Summary of SANS data fitting parameters (\* represents an arbitrary number)

	<b>PA-E3</b>	<b>PA-E3/DBS-COOH (4:1)</b>	<b>PA-E3/DBS-COOH (1:1)</b>	<b>PA-E3/DBS-COOH (1:4)</b>	<b>DBS-COOH</b>
SLD (estimated) / $\times 10^{-6} \text{ \AA}^{-2}$	1.310	1.4874	1.7535	2.0196	2.197
Background / $\text{cm}^{-1}$	$0.021 \pm 0.0002$	$0.013 \pm 0.0002$	$0.014 \pm 0.0002$	$0.016 \pm 0.000$	$0.0129 \pm 0.0001$
Axis ratio	$1.637 \pm 0.015$	$1.53 \pm 0.03$	$1.360 \pm 0.06$	$1.524 \pm 0.10$	
Kuhn Length / $\text{Å}$	$134.0 \pm 2.0$	$111.6 \pm 2.1$	$96.3 \pm 3.15$	$114.4 \pm 9.21$	
Length / $\text{Å}$	2000*	2000*	2000*	2000*	
Radius / $\text{Å}$	$26.9 \pm 0.1$	$27.5 \pm 0.3$	$28.4 \pm 0.7$	$27.9 \pm 1.1$	
Scale (flex ellip cyl)	$1.62 \times 10^{-3} \pm$ $1.155 \times 10^{-5}$	$1.377 \times 10^{-3} \pm$ $1.4096 \times 10^{-5}$	$8.264 \times 10^{-3} \pm$ $2.440 \times 10^{-6}$	$3.962 \times 10^{-4} \pm$ $1.582 \times 10^{-5}$	
Power Law		$2.47 \pm 0.05$	$2.76 \pm 0.04$	$2.947 \pm 0.07$	$2.716 \pm 0.009$
Scale (power law)		$2.054 \times 10^{-5} \pm$ $5.287 \times 10^{-6}$	$1.303 \times 10^{-5} \pm$ $2.825 \times 10^{-6}$	$5.089 \times 10^{-6} \pm$ $1.779 \times 10^{-6}$	$1.183 \times 10^{-5} \pm$ $4.185 \times 10^{-7}$
$\chi^2$	5.936	5.4074	8.5144	2.888	4.6165

## 7. Rheological kinetics for PA-E3, DBS-COOH and PA-E3/DBS-COOH equimolar mixture



**Figure S6.** Time-sweep rheology showing variation of  $\tan \delta$  with time as an evidence of sol-gel phase transition.

## 8. Avrami Equation

By fitting the data acquired through dynamic time sweep rheology into an Avrami model (eq 1 - 4), we were able to gain further insight into the dimensionality of the nanoscale assembly. This model has been applied previously to study the growth kinetics of molecular gelators. The  $X(t)$  represents the volume fraction of the self-assembled (gel) phase, the temperature-dependent constant  $K$  (similar to a rate constant), and the Avrami exponent  $n$  represents the type of growth leading to phase separation.

$$1 - X(t) = \exp(-Kt^n) \quad (1)$$

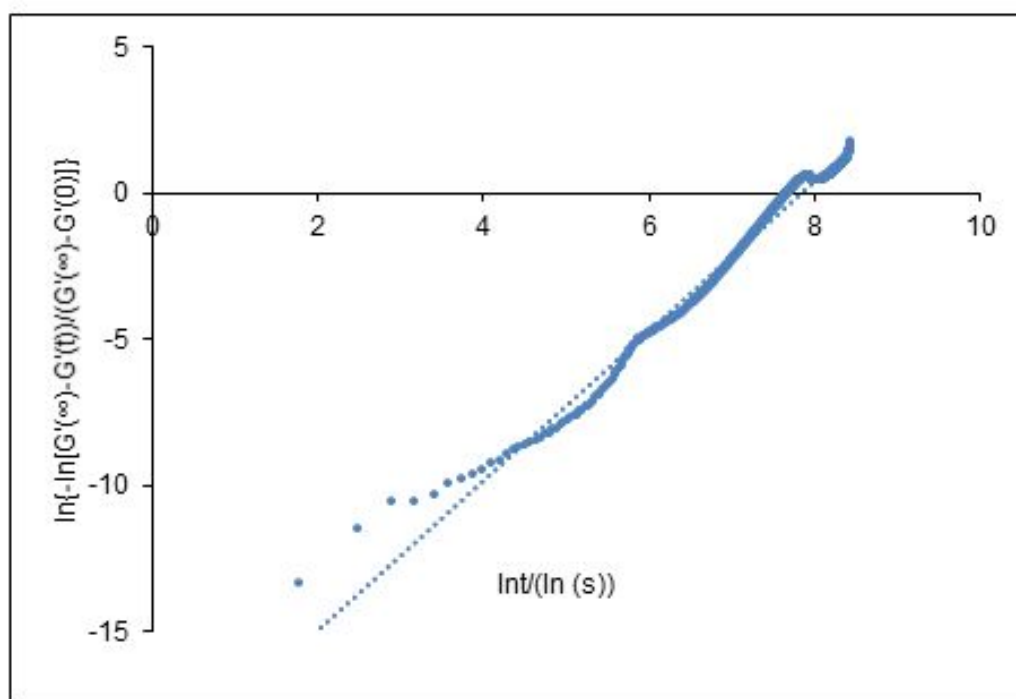
$$\ln(\ln(1/(1 - X(t)))) = \ln K + n \ln(t) \quad (2)$$

$$X(t) = (I(\infty) - I(t)) / (I(\infty) - I(0)) \quad (3)$$

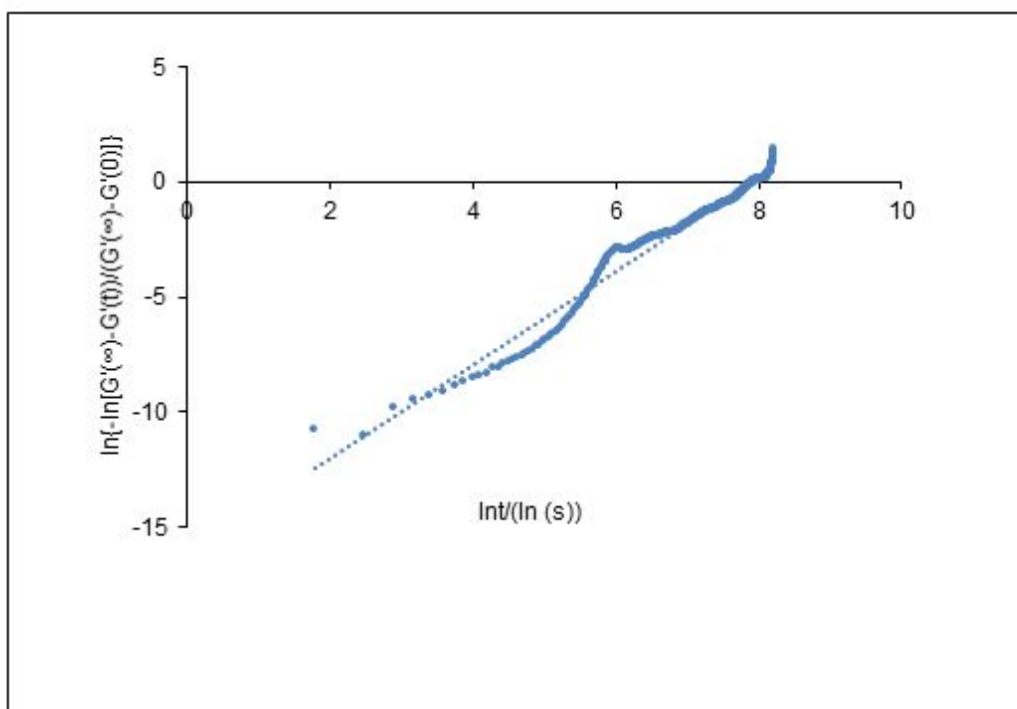
$X(t)$  can be expressed in terms of the experimental values such as  $G'$  values at equilibrium ( $G'(\infty)$ ), at time  $t$  ( $G'(t)$ ) and at the start of the measurement ( $G'(0)$ ). Therefore,

$$G'(t) = (G'(\infty) - G'(t)) / (G'(\infty) - G'(0)) \quad (4)$$

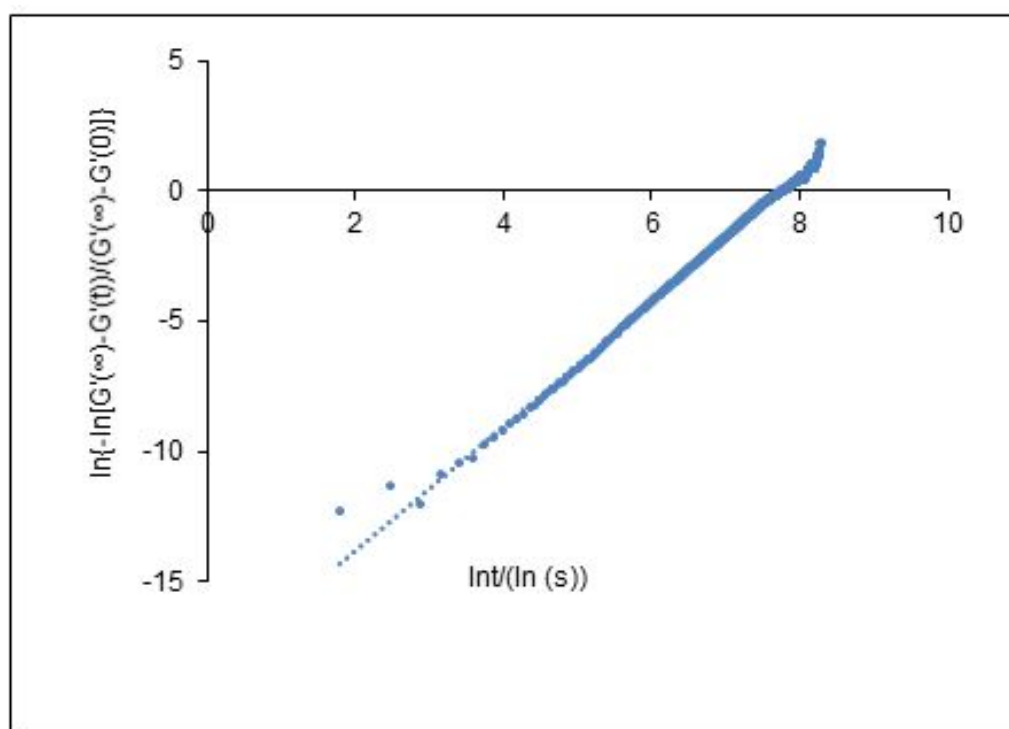
Linear fitting of equation 2 into equation 4 enables the determination of the Avrami exponent  $n$ .



**Figure S7.** Plots of  $G'$  values according to Avrami model for sol-hydrogel phase transition of 10 mM of equimolar mixture of **PA-E3/DBS-COOH** (slope = 2.7,  $R^2 = 0.983$ ).



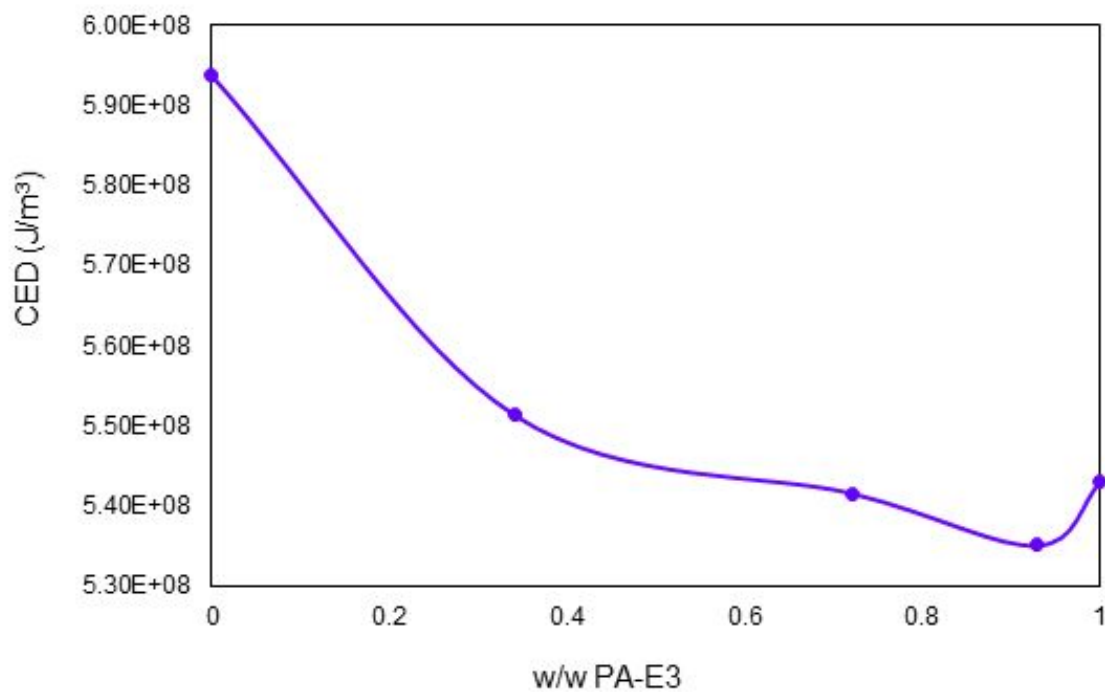
**Figure S8.** Plots of  $G'$  values according to Avrami model for sol-hydrogel phase transition of 10 mM of **PA-E3** (slope = 2.0,  $R^2 = 0.974$ ).



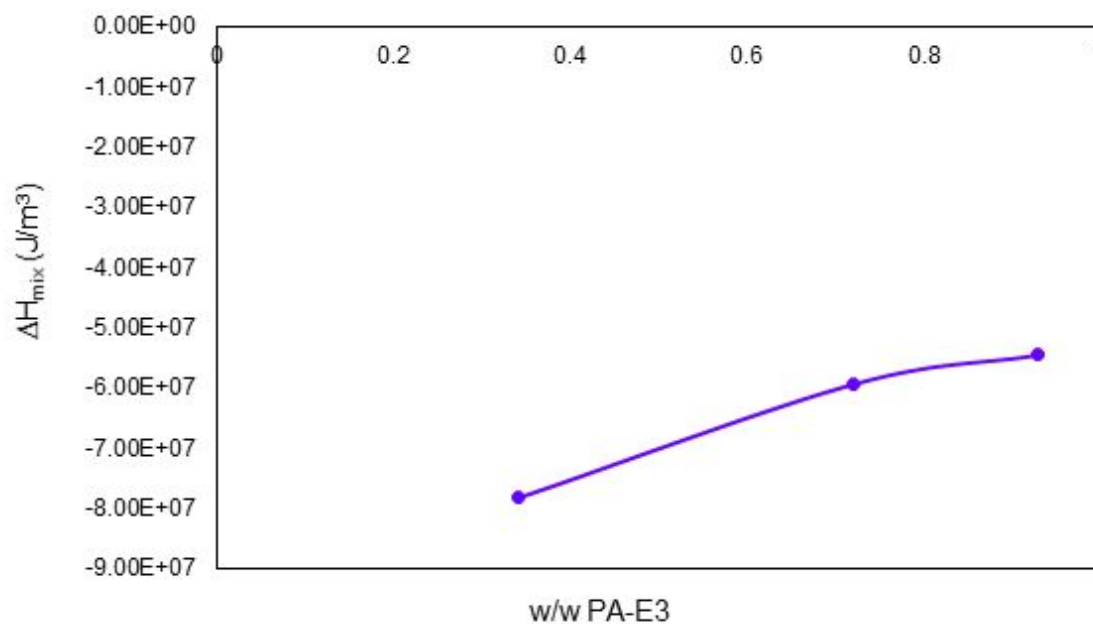
**Figure S9.** Plots of  $G'$  values according to Avrami model for sol-hydrogel phase transition of 10 mM of **DBS-COOH** (slope = 2.2,  $R^2 = 0.996$ ).



## 9. Molecular dynamic simulation details



**Figure S10.** Cohesive energy density (CED) of PA-E3 and DBS-COOH blends

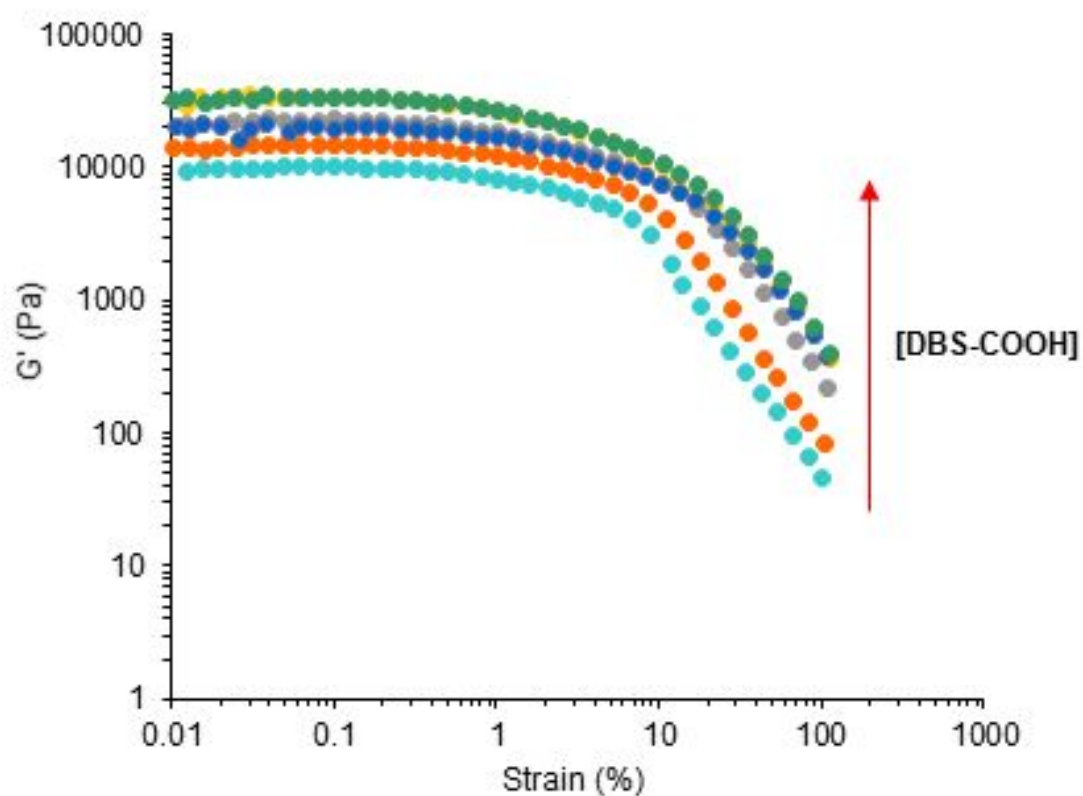


**Figure S11.** Enthalpy of mixing ( $\Delta H_{\text{mix}}$ ) of PA-E3 and DBS-COOH blends.

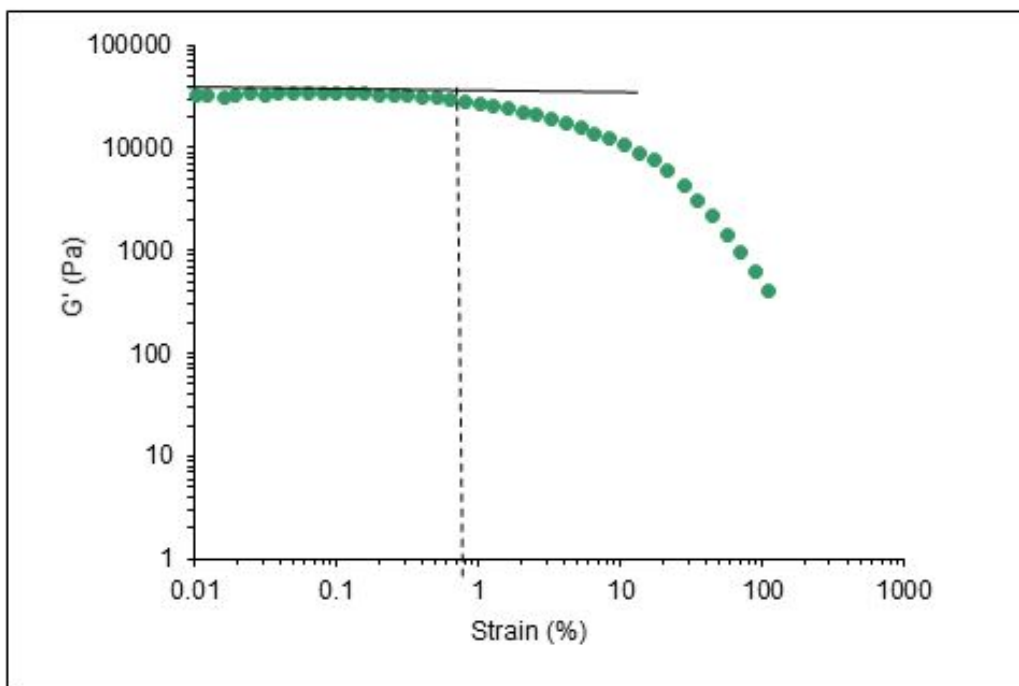
**Table S2.** Summary of intermolecular interaction distances between **PA-E3, DBS-COOH** and their co-assemblies.

Interaction between	Interaction distance (Å)
O_carbonyl of DBSCOOH and H-amide of E3	1.58
O_carbonyl of DBSCOOH and H_carboxyl of E3	1.73
O5 of DBSCOOH and H-amide of E3	2.10
O6 of DBSCOOH and H-amide of E3	2.00

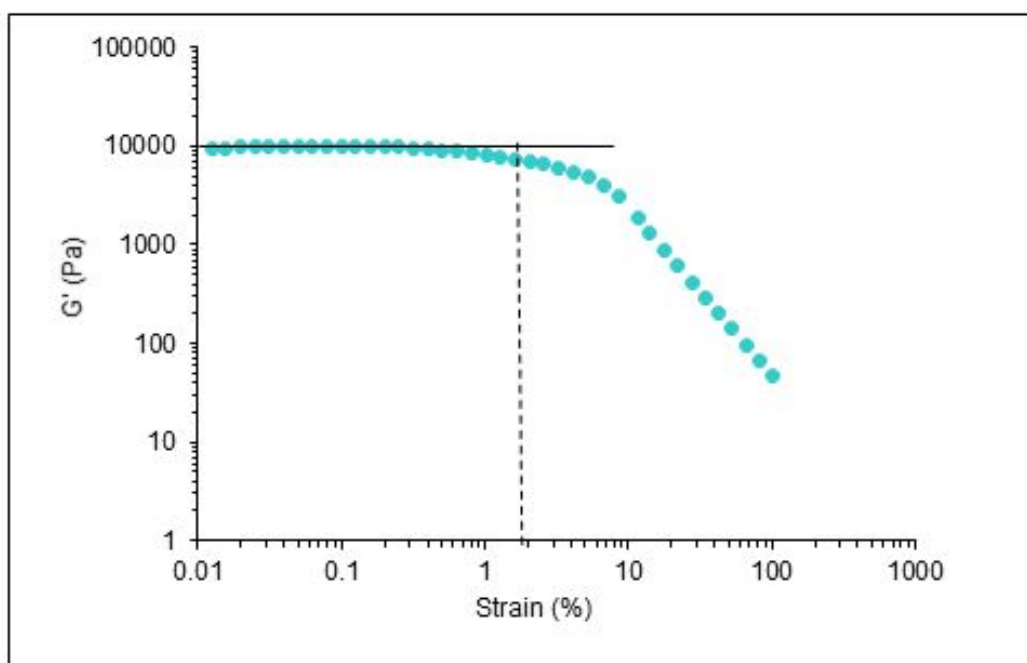
**10. Amplitude sweeps, critical strain and self-recovery of hydrogels**



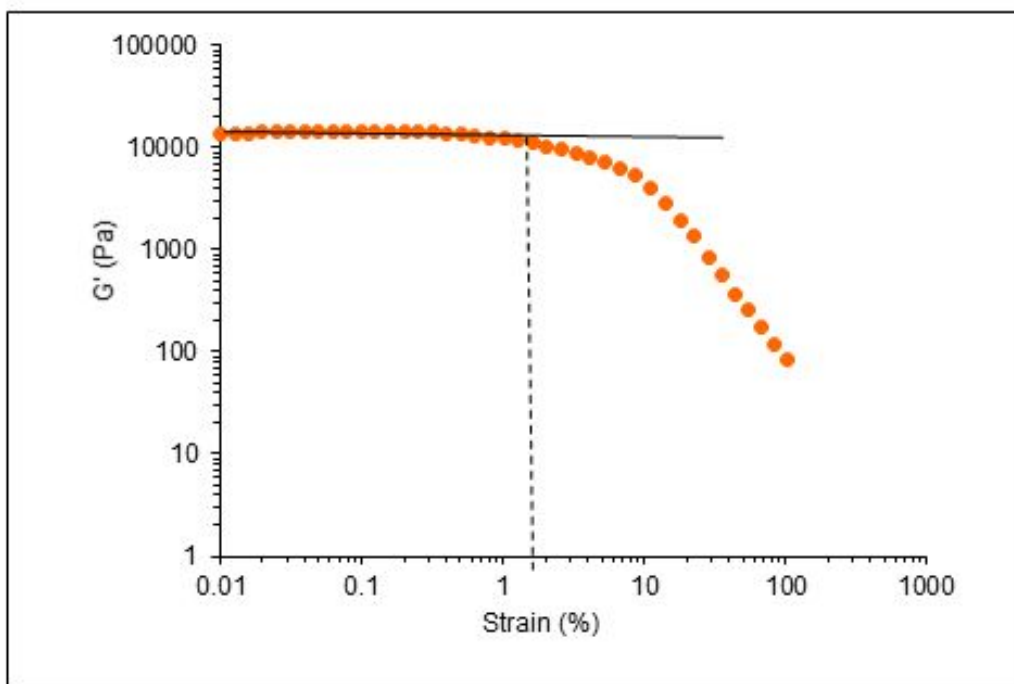
**Figure S12.** Amplitude sweep rheographs showing increasing storage modulus with concentration of **DBS-COOH**.



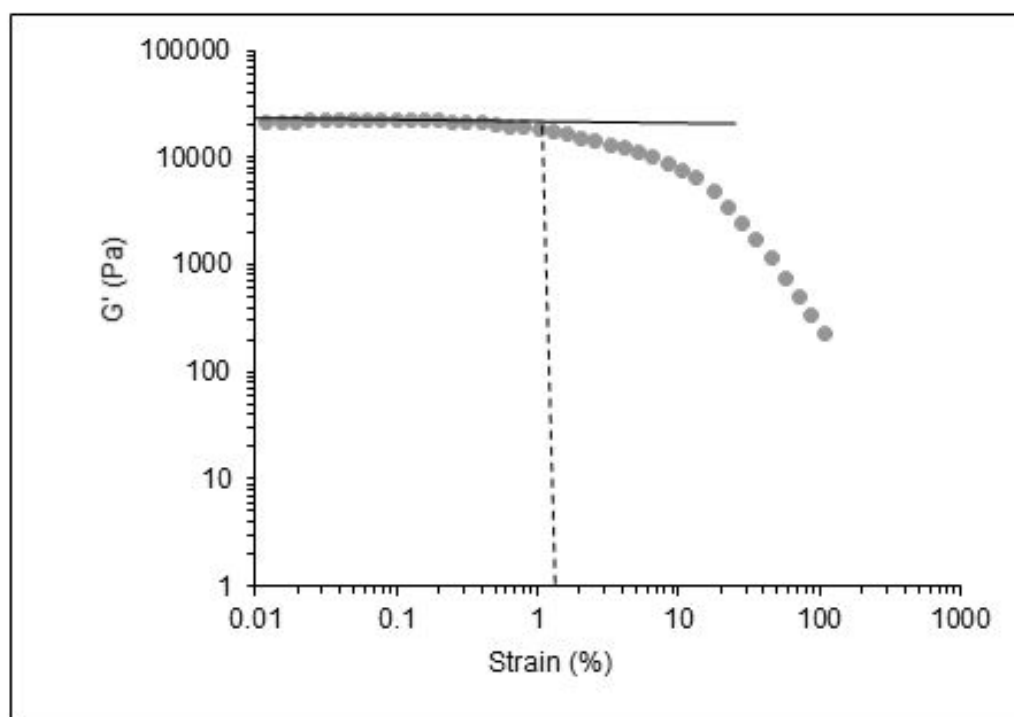
**Figure S13.** Critical strain of pure **DBS-COOH** hydrogel.



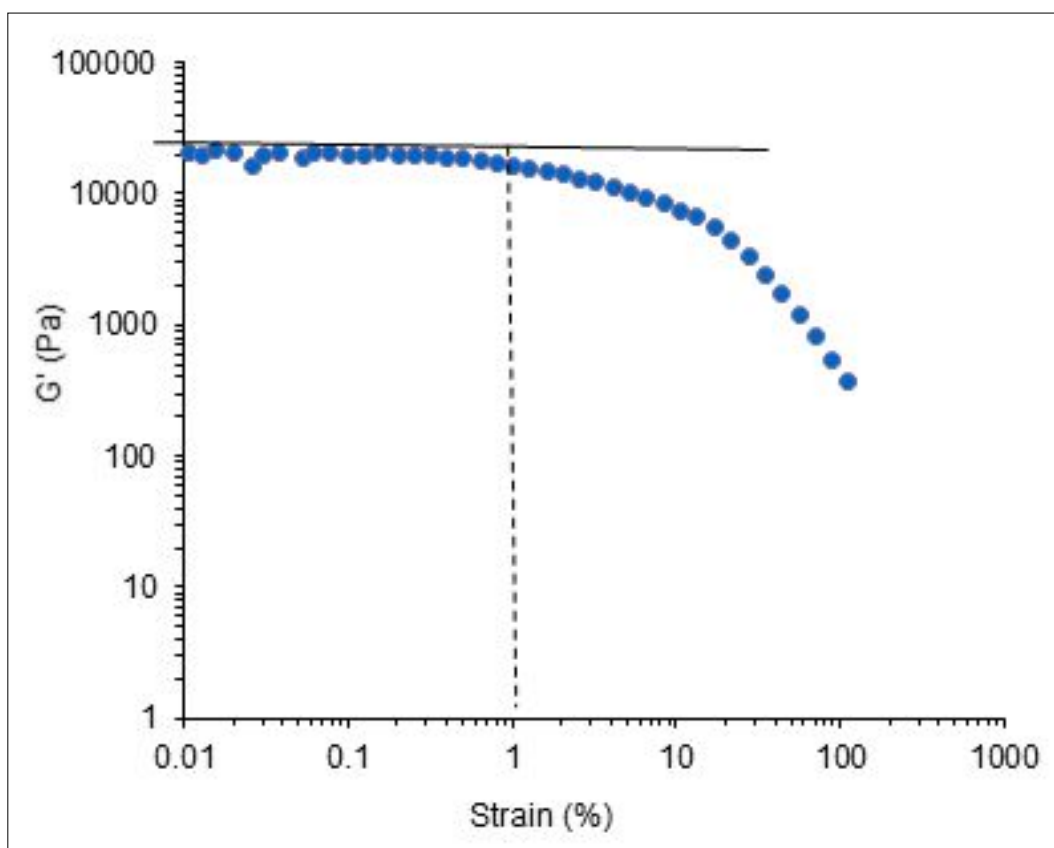
**Figure S14.** Critical strain of pure **PA-E3** hydrogel.



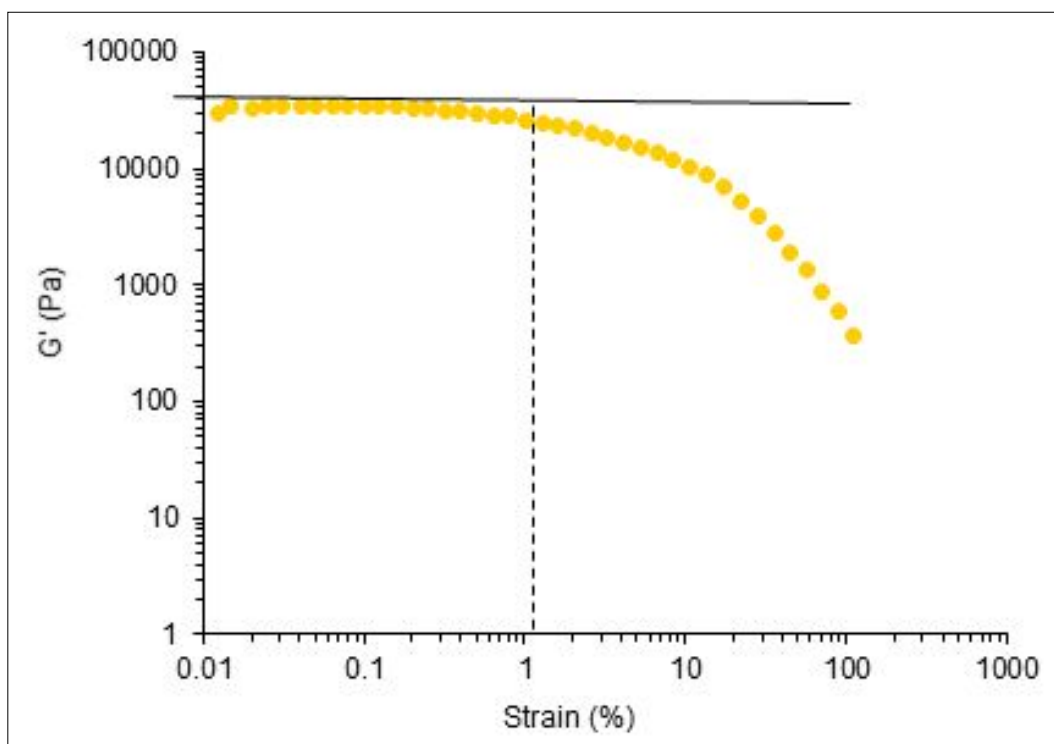
**Figure S15.** Critical strain of 80% **PA-E3** + 20% **DBS-COOH** hydrogel.



**Figure S16.** Critical strain of 60% **PA-E3** + 40% **DBS-COOH** hydrogel.

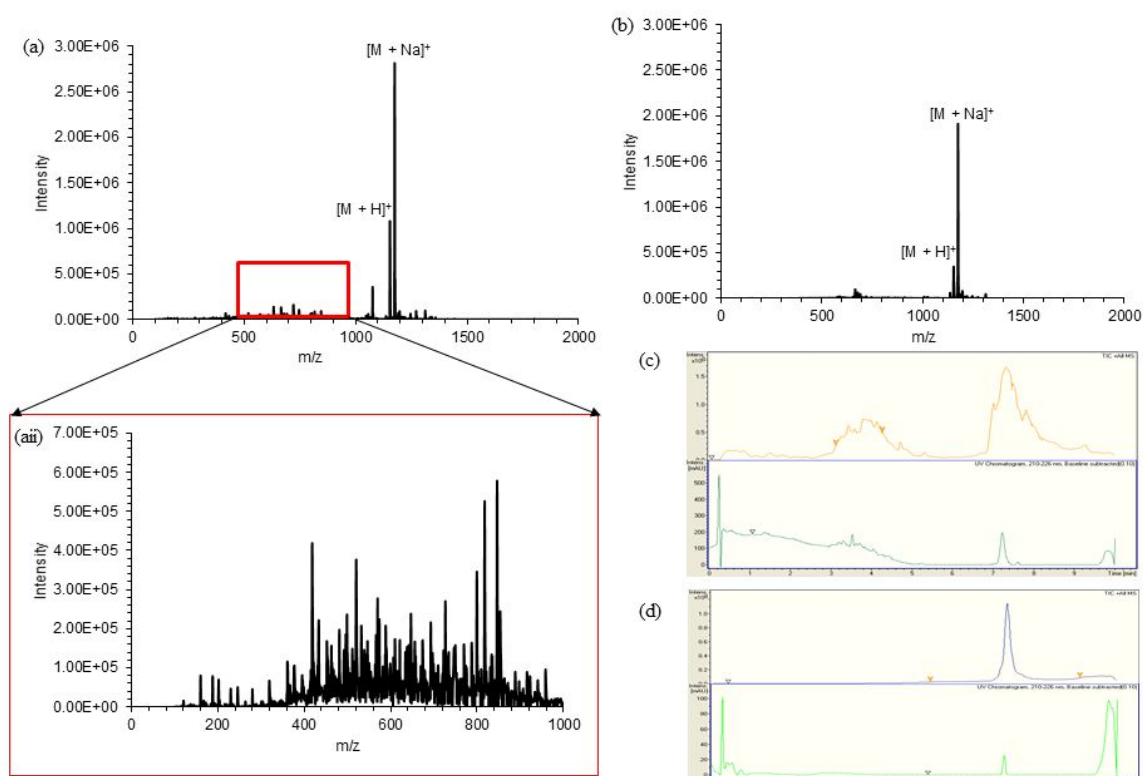


**Figure S17.** Critical strain of 40% PA-E3 + 60% DBS-COOH hydrogel.



**Figure S18.** Critical strain of 20% PA-E3 + 80% DBS-COOH hydrogel.

## 11. Characterizing proteolytic stability of the hydrogels with mass spectrometry



**Figure S19.** LC-MS for a mixture of (a and aii) 0.1 mM PA-E3/proteinase K (5 mg/mL), (b) 0.1 mM PA-E3/0.1 mM DBS-COOH/proteinase K (5 mg/mL), (c) TIC and UV trace for 0.1 mM PA-E3/proteinase K (5 mg/mL) and (d) 0.1 mM PA-E3/0.1 mM DBS-COOH/proteinase K (5 mg/mL).

## References

- (1) Mata, A.; Palmer, L.; Tejeda-Montes, E.; Stupp, S. I. In *Nanotechnology in Regenerative Medicine: Methods and Protocols*; Navarro, M.; Planell, J. A., Eds.; Humana Press: Totowa, NJ, 2012, DOI:10.1007/978-1-61779-388-2\_3 10.1007/978-1-61779-388-2, pp. 39-49.
- (2) Cornwell, D. J.; Okesola, B. O.; Smith, D. K. Hybrid polymer and low molecular weight gels – dynamic two-component soft materials with both responsive and robust nanoscale networks. *Soft Matter* 2013, **9**, 8730-8736.