Electronic Supplementary Material (ESI) for Soft Matter.

Supporting Information for: **Multi-step control over self-assembled hydrogels of peptide-derived building blocks and a polymeric cross-linker**

Van Duc Nguyen[a], Asish Pal[b], Frank Snijkers[a], Mathieu Colomb-Delsuc[b], Giulia Leonetti[b], Sijbren Otto*[b], and Jasper van der Gucht*[a]

[a]Physical Chemistry and Soft Matter, Wageningen University
Dreijenplein 6, 6703 HB Wageningen (The Netherlands)
[b]Centre for Systems Chemistry, Stratingh Institute, University of Groningen
Nijenborgh 4, 9747 AG Groningen (The Netherlands)

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Ultra performance liquid chromatography (UPLC) analyses.

UPLC analyses were performed on a Waters Acquity H-class machine equipped with diode array UV/Vis detector. The eluents, acetonitrile and water contained 0.1% of TFA. The peptide solutions were eluted with a gradient as shown in detail in Table S1 and Fig. S1.

Table S1: Solution components eluted using acetonitrile and water containing 0.1 % of TFA

Time (min)	% of Acetonitrile	% of Water
0	10	90
1	10	90
1.3	25	75
3	28	72
11	40	60
11.5	95	5
12	95	5
12.5	10	90
17	10	90

Nuclear Magnetic Resonance spectometry (H-NMR) analyses.

The final product of triblock copolymers was dissolved in deuterium oxide to determine the structure by ^{1}H-NMR on a Bruker Avance III 400 MHz Nuclear Magnetic Resonance (NMR) spectrometer. The measured ^{1}H-NMR spectrum of the triblock copolymer is shown in Fig. S2. Following Lemmers *et al.* [1], we determine the degree of polymerization (DP) by the ratio of the integrals of peak 1 to the one of PEG, taking into account the number of protons in a PEG monomer and group 1 of the triblock copolymer: $DP = 2I_1 N_{PEG}/I_{PEG} = 50$, with I_1 the integral surface of the group 1-peak (see [1] for details), I_{PEG} the surface of the PEG-peak, and N_{PEG} the number of PEG-monomers in each chain. Hence we end up with a triblock with a neutral mid-block and two negatively charged end-blocks: $PSPMA_{25}$-PEO_{230}-$PSPMA_{25}$.

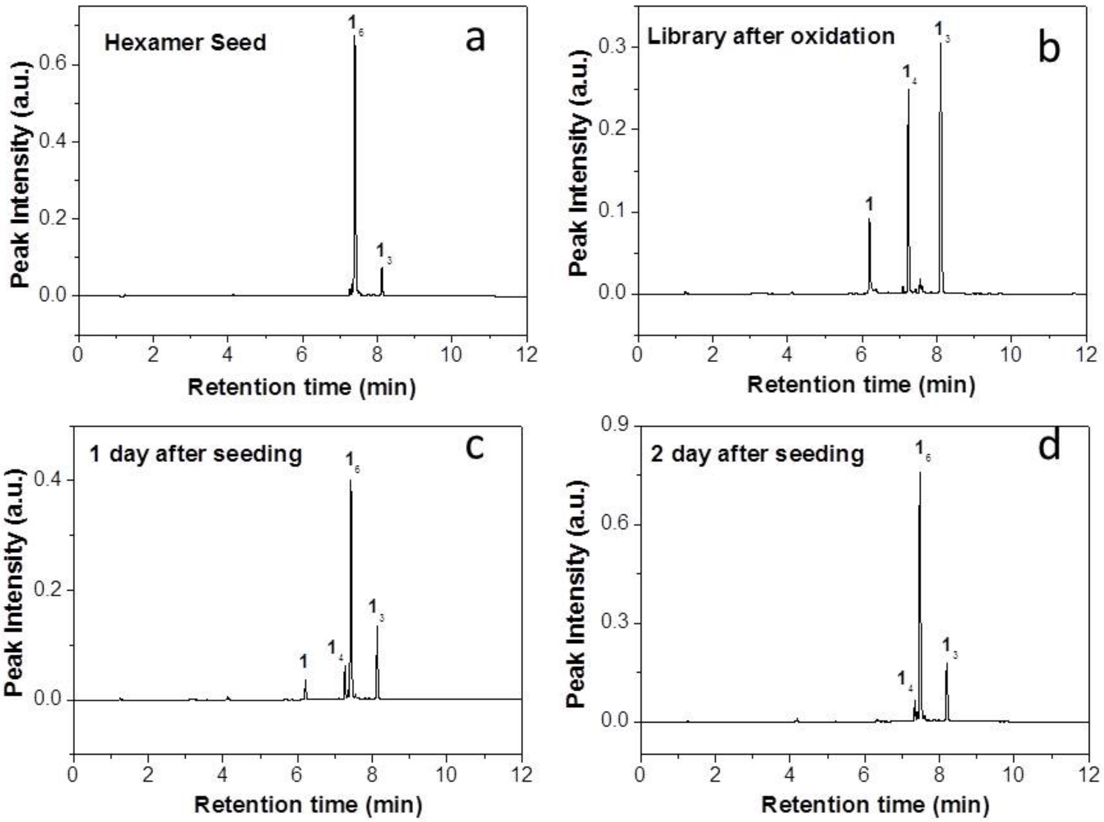

Figure S1: **UPLC traces for:** (a) pre-formed hexamer seed, (b) 3.8 mM solution of **1** after oxidation by sodium perborate solution, (c) 1 day and (d) 2 days after 20 % seeding by hexamer seed.

Volume fraction and entanglement length estimations

Volume fraction of $\mathbf{1}_6$ is estimated using $\phi = \pi \rho a d^2/4$ [2], where $a = 0.48$ nm, and $d = 7$ nm are the length per monomer and diameter of $\mathbf{1}_6$, respectively. The number density of $\mathbf{1}_6$ $\rho = 3.8 \times 10^{23}$ m^{-3} is estimated using $\rho = cN_A$, where $c = 0.63$ mole/m^3 the $\mathbf{1}_6$ concentration, and $N_A = 6.02 \times 10^{23}$ Avodgadro's number. Inserting these numbers to the above equation, we obtain $\phi = 6.7 \times 10^{-3}$.

From this, we obtain the entanglement length as $L_e \simeq (L_P)^{1/5}(a\rho)^{-2/5}$ [3] with L_P =1.5 μm the measured persistence length. This gives $L_e \simeq 135$ nm.

References

[1] M. Lemmers, J. Sprakel, I. K. Voets, J. V. D. Gucht, and M. A. C. Stuart, "Multireseponsive reversible gels based on charge-driven assembly," *Angewandte Chemie*, vol. 49, pp. 708–711, 2010.

[2] D. C. Morse, "Viscoelasticity of concentrated isotropic solutions of semiflexible polymers. 1. model and stress tensor," *Macromolecules*, vol. 31, pp. 7030–7043, 1998.

[3] F. MacKintosh, J. Kas, and P. Janmey, "Elasticity of semiflexible biopolymer networks," *Physical review letters*, vol. 75, p. 4425, 1995.

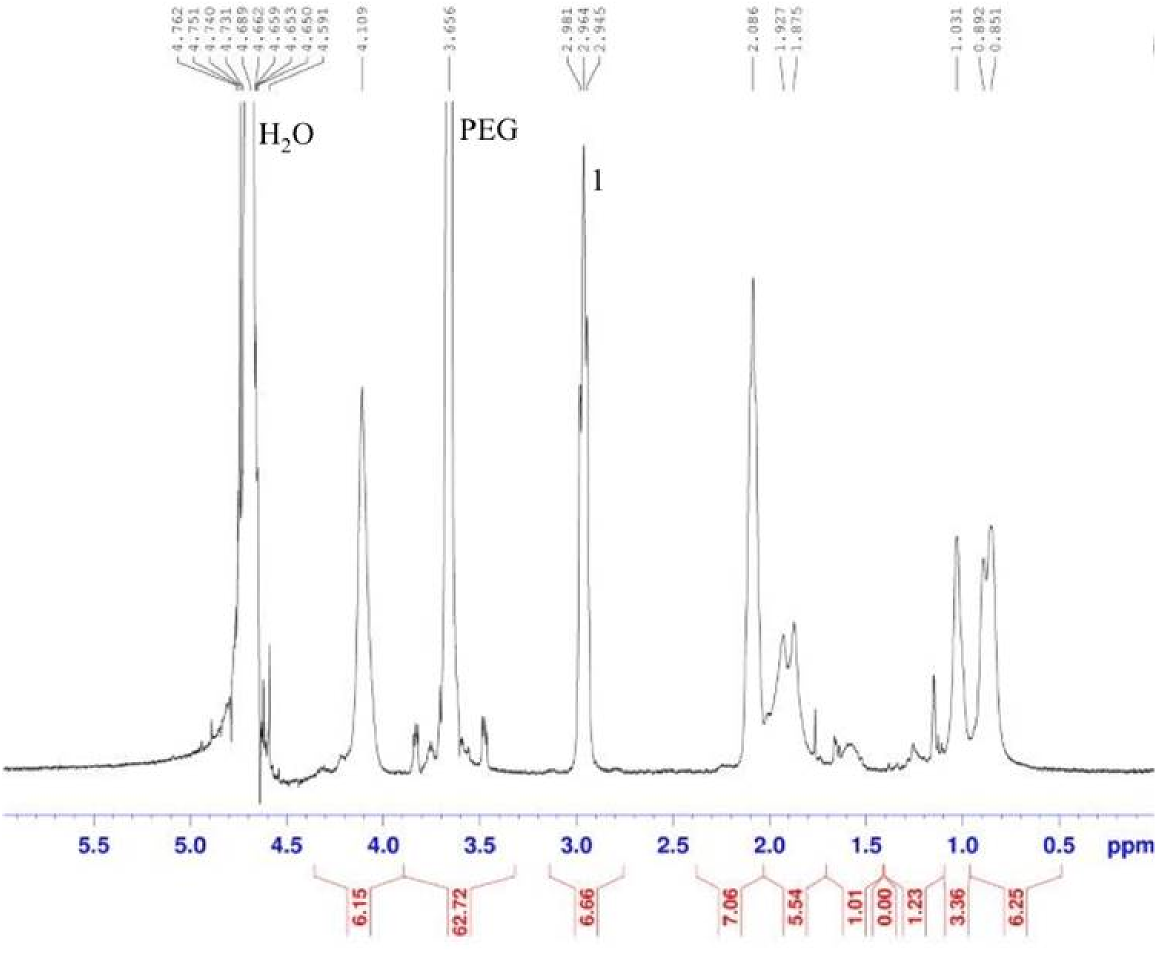

Figure S2: ^{1}H-NMR-spectrum of the negatively charged triblock copolymer.