

Supplemental Data:

Thermosensitive Double network of Zwitterionic polymers for controlled mechanical strength of hydrogel

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Experiment section

2.1 Materials

2-Methacryloyloxyethyl phosphorylcholine, (MPC) 97%, [2-(Methacryloyloxy) ethyl] dimethyl-(3-sulfopropyl) ammonium hydroxide (DMAPS) 97%, N, N'-Methylenebis(acrylamide) 99%, α -Ketoglutaric acid were purchased from Sigma-Aldrich. Customized transparent container for UV initiated polymerization cells was made by polycarbonate using a 3D printer (Ultimaker 2⁺).

2.2 Polymer synthesis

The polymeric hydrogels were synthesized by UV-initiated free radical polymerization, in

which initiated by α -Ketoglutaric acid (α -KA) as an initiator and under UV light (365nm, 4 watt, 0.16 amps, 115V-60Hz) after purged by nitrogen gas into solution for 10 minutes. Monomer concentration (C_m) in total (either MPC or DMAPS) is 1.0 M, representing as $C_m = \text{MPC monomer } C_{[\text{MPC}]} + \text{DMAPS monomer } C_{[\text{DMAPS}]}$ in the end. The concentration of N, N'-Methylenebis (acrylamide) as chemical crosslinker and α -KA are 0.2 mol% and 0.1 mol% (both relative to C_m), respectively. Two-step synthesis was prepared for double network hydrogel. In the first step, the polymer was prepared from either MPC or DMAPS to form homo-polymers. The crude products were precipitated in acetone (v/v 1:50) for purification. In the second step, the other monomer (either DMAPS or MPC) and the chemical crosslinker were added and mixed with the first polymer products to form the double network hydrogel matrix, as shown in **Figure 1** and **Figure S4**.

In order to easily control gel shape for characterization, the second step to form hydrogel took place in 3D-printed polycarbonate cells (18 mm \times 18 mm \times 5 mm). These customized and transparent cells can allow UV-initiated polymerization and stably produce hydrogel samples for further measurements (**Figure S1**).

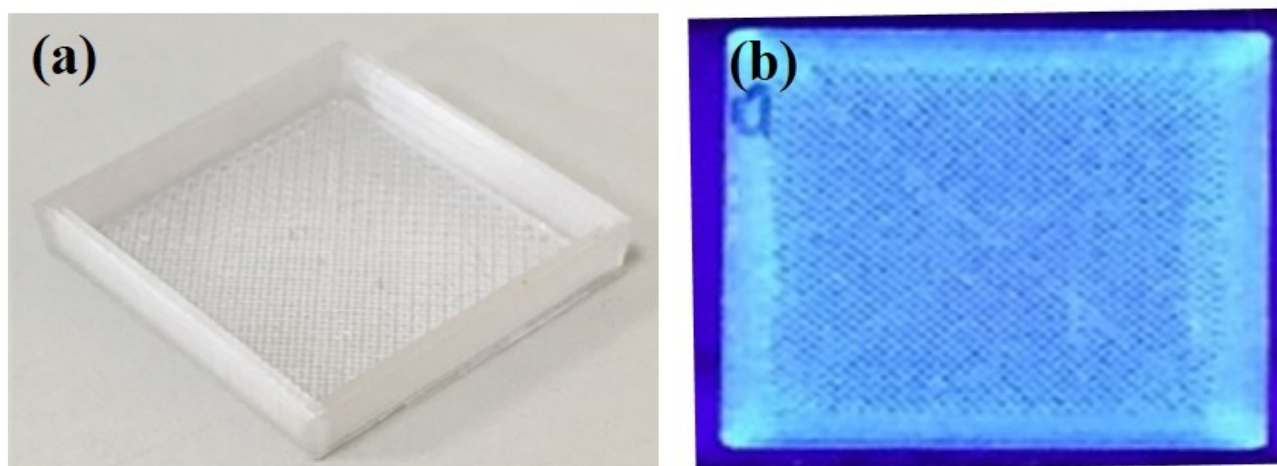


Figure S1. Photographs of 3D-printed polycarbonate (PC) holder for UV-polymerization to form DN hydrogel.

2.3 Polymer characterization

Nuclear magnetic resonance (NMR) measurements were performed on a ^1H nuclear magnetic resonance spectra, Bruker Avance 600 MHz NMR spectrometer. The concentration of sample 1 wt% were dissolved in D_2O for the NMR measurements. The molecular weights and molecular-weight distributions of the polymers were determined using aqueous-phase gel permeation chromatography (GPC) with a commercial standard of Pullulan. For minimizing the aggregation in the columns, the polymers were dissolved in phosphate buffered saline (PBS) and the eluent was chosen the same buffer with 1 mL min^{-1} .

2.4 Raman measurement

Raman spectra was performed via Laser Raman Spectrometer, NRS-5100. Samples were used powder or bulk hydrogel, exposure 10 sec, accumulation 10 times, and laser wavelength 543 nm, Optical Density (OD) 6. For the 2D mapping, a random-selected zone in each section ($X \times Y = 50 \times 50 \mu\text{m}^2$) of bulk hydrogel was measured with an internal $5 \mu\text{m}$, and in the 3D mapping, each scanning section was a three-dimensional zone of $X \cdot Y \cdot Z = 10 \times 10 \times 10 \mu\text{m}^3$ and with the internal distance, $1 \mu\text{m}$ between each section.

2.6 Rheological viscosity measurement

The viscoelastic properties of the hydrogels were studied in aqueous solutions, using a stress-controlled rheometer (Modular Compact Rheometer, Anton Paar MCR-102) with a constant geometry (produced by the 3D-printed cells as mentioned above). Swelling-state of samples were prepared by a hydrogel immersed in water one day before and swiped the excess water from the surface before measurement. The rheological viscosities were measured under linear viscoelastic regime, which was established by a stress sweep at different frequencies (ramp shear rate) and temperatures. The other parameters were fixed at constant shear rate ($1 /\text{s}$), surface force (1 N), and viscosities (η^*) were recorded between $20 \text{ }^\circ\text{C}$ and $80 \text{ }^\circ\text{C}$ with either heating or cooling rates of 5

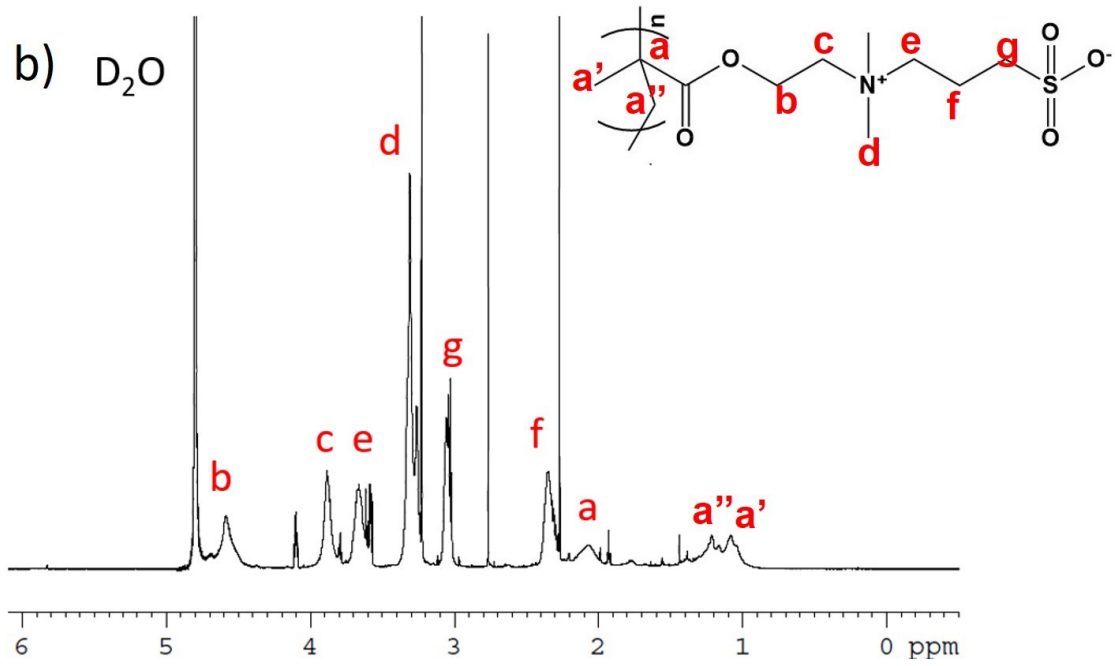
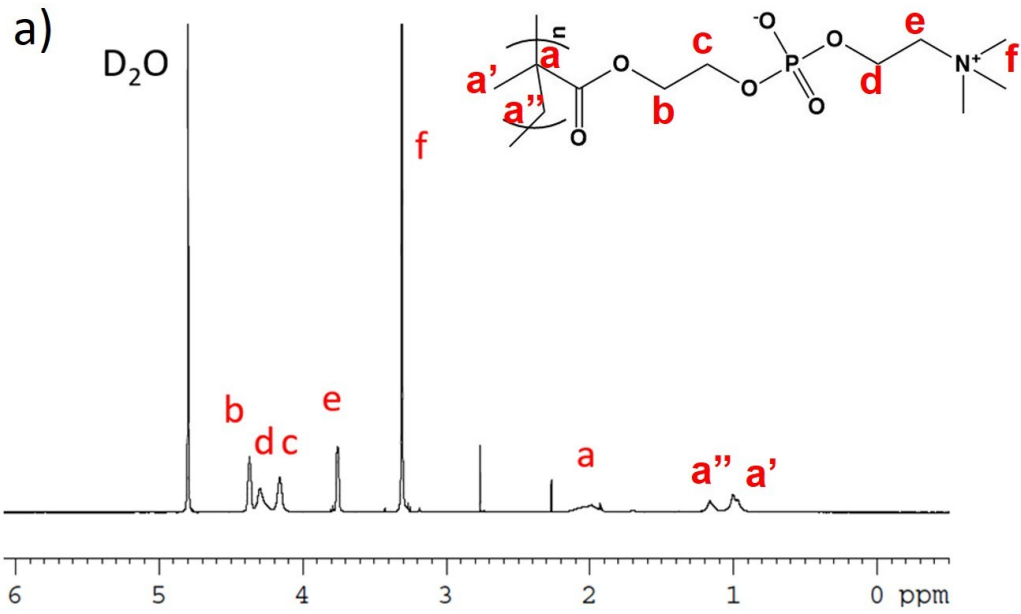
°C/min.

2.2.7 Turbidity measurements

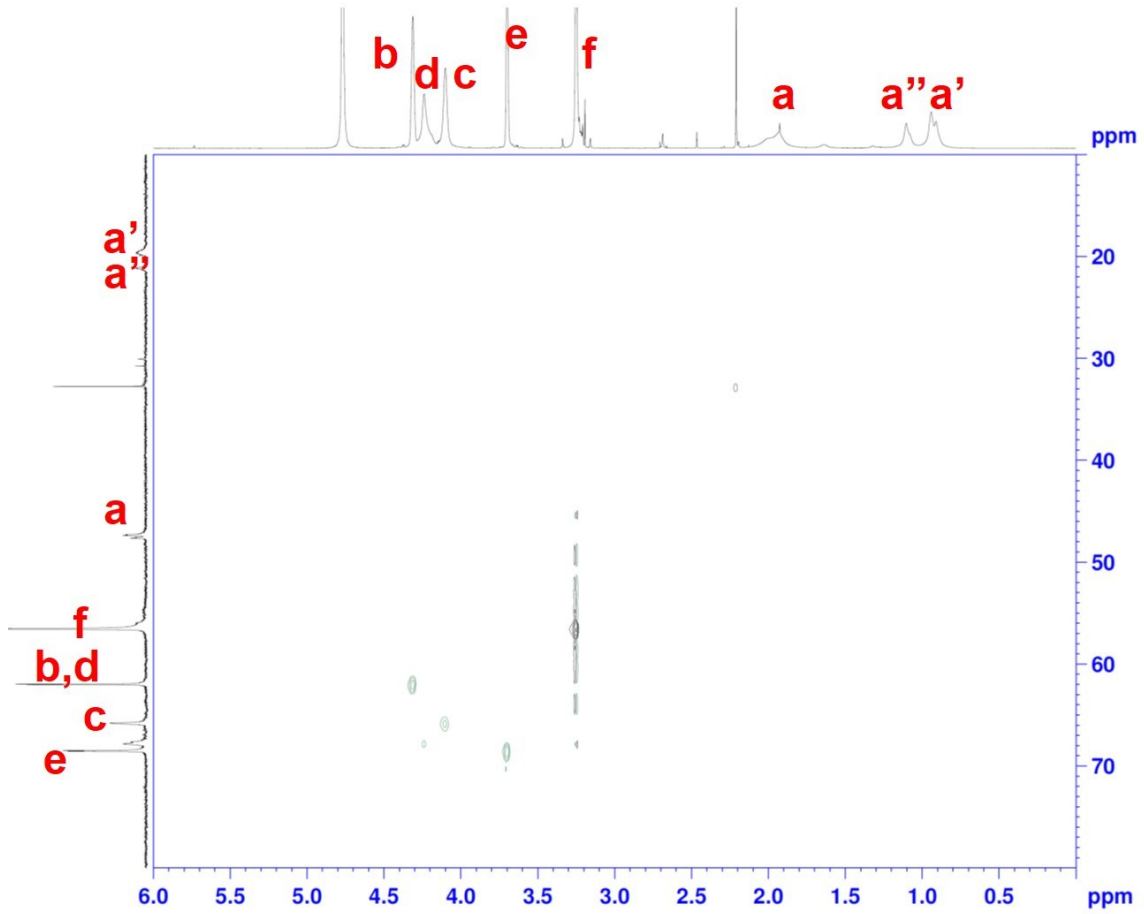
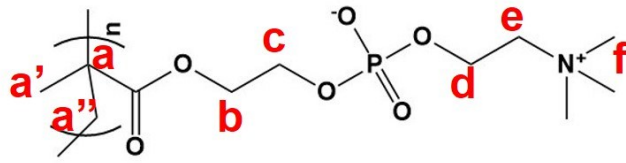
Turbidity was measured by UV-Vis spectra with the wavelength of 550 nm. Firstly, the polymers were dissolved in pure water at different concentrations (1, 5, 10 wt%). After that, the polymer solution was measured by light absorption at different temperatures. The temperatures of samples initialized from highest temperature 80 °C and gradually reduced to 20 °C with a cooling rate (5 °C/min) [1]. The UCST transition temperature was considered as 50 % of visible light transmittance.

2.2.8 Morphology studies

Morphology were studied by Scanning Electron Microscopy (LV SEM). Samples were prepared from bulk hydrogel and frozen by liquid nitrogen, then dried by freeze dryer (Speed Trup). The dried samples were coated by platinum on the surface before SEM measurement.



c)



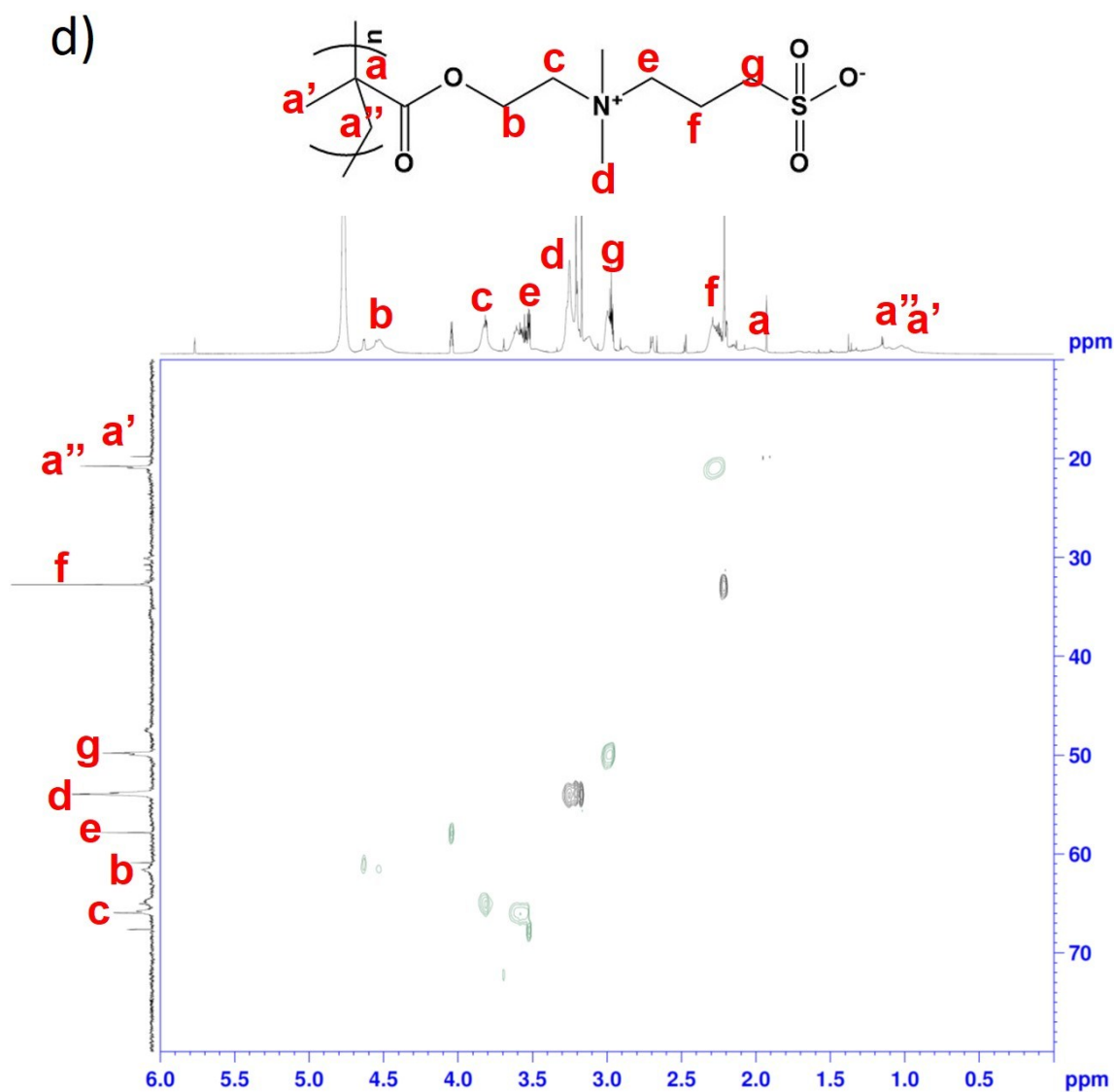


Figure S2. ^1H NMR spectrum of (a) poly-MPC and (b) poly-DMAPS. HSQC (^1H v.s. ^{13}C) 2D-spectrum of (c) poly-MPC and (d) poly-DMAPS in D_2O at the room temperature.

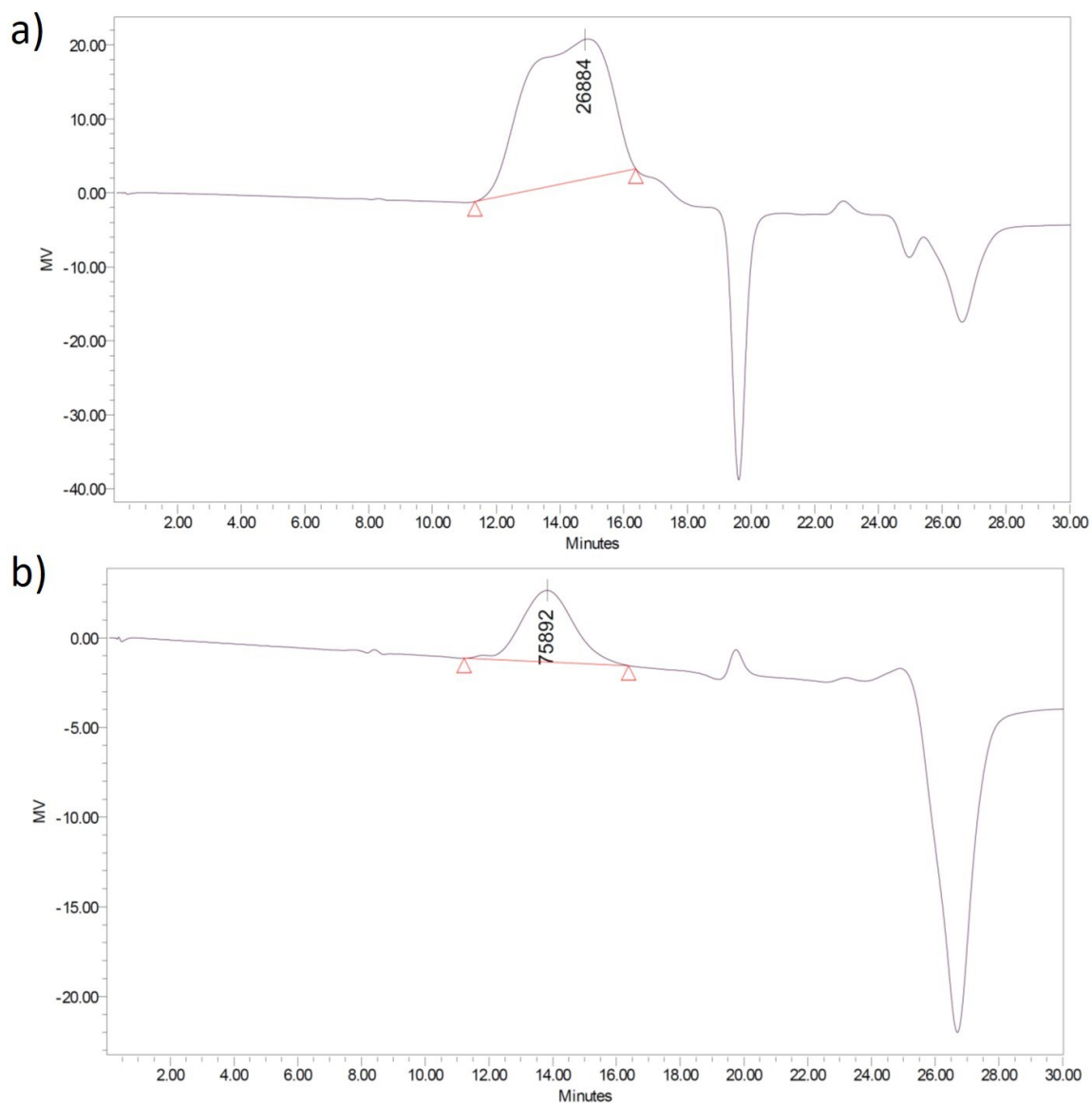


Figure S3. Measurement of relative molecular weight of poly-DMAPS via gel permeation chromatography (GPC) with elute phase PBS 1 mL/min. The calibrated by a commercial standard Pullulan.

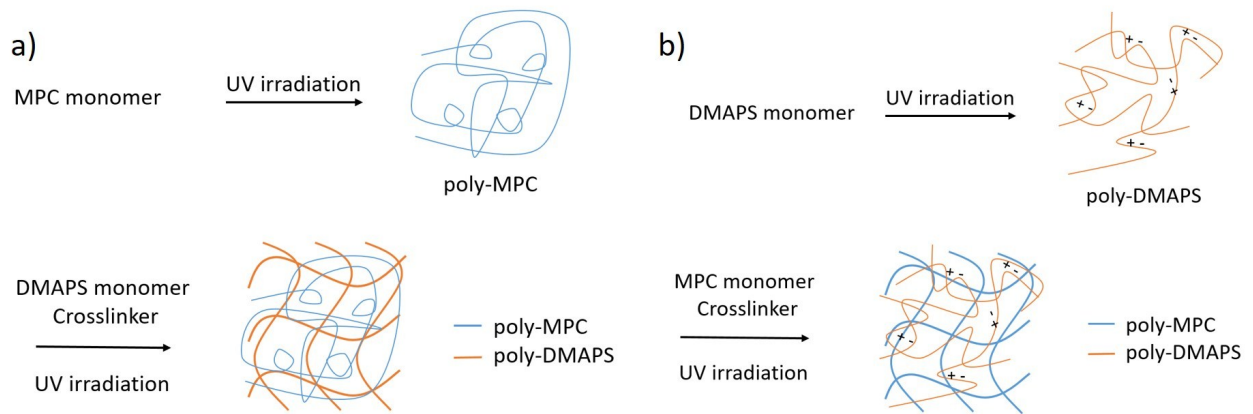


Figure S4. Double-penetrated network hydrogel schemes. a) Polymerization of prepared poly-MPC and DMAPS monomers with di-acryl crosslinker to form double network hydrogel (MD gel). (b) Polymerization of prepared poly-DMAPS and MPC monomers with bis-acryl terminal crosslinker to form double network hydrogel (DM gel).

Reference

1. Karjalainen, E., V. Aseyev, and H. Tenhu, *Upper or lower critical solution temperature, or both? Studies on cationic copolymers of N-isopropylacrylamide*. Polymer Chemistry, 2015. **6**(16): p. 3074-3082.