Supporting Information for

Upper Critical Solution Temperature (UCST) Behavior of Pyroglutamate-Functionalized Non-Ionic Homopolymers: Cosolvency in Water/Alcohol Binary Solutions

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Experimental section

Materials: 2-Hydroxyethyl methacrylate (HEMA, 97%, Aldrich), 4-vinylbenzyl chloride (VBC, 90%, Aldrich), anhydrous *N*,*N*-dimethylformamide (DMF, 99.9%, Aldrich), L-pyroglutamic acid (PGA, 99%, Sisco Research Laboratories Pvt. Ltd. (SRL)) dicyclohexylcarbodiimide (DCC, 99%, SRL), 4-(dimethylamino)pyridine (DMAP, extra pure, SRL), potassium carbonate (K_2CO_3, 99%, Merck), urea (extra pure, SRL), sodium thiocyanate (NaSCN, ≥ 98.5%, Merck), sodium sulphate (Na_2SO_4, anhydrous, 99.5%, SRL), sodium chloride (NaCl, 99%, Merck) were used as received from commercial sources. The initiator 2,2′-azobis-(2-methylpropionitrile) (AIBN, Sigma, 98%) was recrystallized twice from methanol before being used in polymerization reactions. For the analysis of thermoresponsive features of polymers, HPLC water (SRL), methanol (anhydrous, Sigma), ethanol (anhydrous, Sigma), and isopropanol (SRL) were used as received. The synthesis of 4-cyano-(dodecylsulfanylthiocarbonyl)sulfanylpentanoic acid (CDP) was done by following the standard literature procedure.[1] The NMR solvents, DMSO-d_6 (99% D) and $CDCl_3$ (99.8% D), were purchased from Cambridge Isotope Laboratories, Inc., USA. Dichloromethane (DCM), hexane (a mixture of isomers), acetone, methanol, ethyl acetate, diethyl ether, and chloroform were bought from Merck and used as received.

Instrumentation: ^1H NMR spectroscopic measurements were carried out using a 500 MHz Bruker Avance III NMR spectrometer at 25 °C using tetramethylsilane (TMS) as an internal standard. High-resolution mass spectrometry (HRMS) of the monomer was performed in Waters XeVO G2-XS QTof instrument. Before the experiment, the samples were prepared in methanol, and filtered through a 0.45 μm polytetrafluoroethylene (PTFE) filter. Size exclusion chromatography (SEC) was performed in DMF solvent at 40 °C at 0.8 mL/min flow rate. The SEC instrument contained a Waters 2414 refractive index (RI) detector, a Waters 1515 HPLC pump, one PolarGel-M guard column (50 × 7.5 mm), and two PolarGel-M analytical columns (300 × 7.5 mm). The instrument was calibrated against polystyrene (PS) standards for both the homopolymers. The molecular weights and dispersity (*Đ*) values were calculated from the calibration graph using Empower software. Before performing SEC, polymer solutions were filtered through a 0.45 μm syringe filter. Turbidity measurements were performed with Perkin Elmer Lambda 35 UV-Vis spectrophotometer using a Peltier control and a cooling unit. Field emission-scanning electron microscopy (FESEM) study was performed on a Carl Zeiss Sigma FESEM instrument. The imaging of coacervate droplets was carried out by using a polarized optical microscopic imaging unit from Byan Olympus, equipped with a polarizer and a CCD camera at 40X magnification. Epiflorescence microscopic images were recorded in an Olympus IX-81 instrument in the liquid state to observe the bright field imaging of coacervate droplets. For estimating the Nile red encapsulation efficiency inside the **PHEPGA1** coacervate, Horiba JobinYvon (Fluoromax-3, Xe-150 W, 250-900 nm) fluorescence spectrophotometer was used.

Turbidity measurements: Turbidimetry was employed to determine the cloud point temperature (T_{cp}) associated with the phase transition behavior.[2] A UV-Vis spectrophotometer equipped with a Peltier temperature control unit was used to monitor the transmittance of 0.2 wt% solutions of **PHEPGA** and **PVBPGA** in alcohol-water binary mixtures, contained within 1.0 mL quartz cuvettes. Measurements were conducted at a constant wavelength of 600 nm over a temperature range of 20-60 ^oC, with the temperature being adjusted in 2 ^oC increments. Each reading was taken after allowing the system to equilibrate for approximately 5 min. T_{cp} was defined as the temperature at which the transmittance of the polymer solution reached 50% of its maximum value. The UCST-type transitions were monitored during the cooling process from a pre-heated state.

Coacervate preparation: A 1 wt% stock solution of **PHEPGA1** was prepared in a 20% ethanol-water mixture and kept at room temperature below its cloud point temperature for 12 h to allow phase separation to occur. Following this period, the supernatant was cautiously separated from the polymer-rich phase (coacervate). The dense polymer-rich coacervate phase was then employed for analyzing droplet formation using polarized optical microscopy, epifluorescence microscopy, and FESEM analysis.

*Encapsulation of Nile red by **PHEPGA1** coacervate:* For estimating the encapsulation capability of **PHEPGA1** coacervate prepared in a 20% ethanol-water mixture, 10 μL of Nile red (as a hydrophobic dye, 1 mg/mL) was added to obtain a final polymer concentration of 10 mg/mL.[3] Encapsulated polymer solutions were kept at room temperature (below cloud point temperature) for 12 h until complete liquid-liquid phase separation was observed. To assess Nile red encapsulation efficiency within the coacervate, the **PHEPGA1** polymer-rich phase was centrifuged at 10,000 rpm at 25 °C. The unencapsulated material was eliminated by rinsing the samples three times with deionized water. The resulting supernatants were collected, and the amount of encapsulated Nile red was determined using fluorescence spectroscopy. The Nile red loading efficiency (LE = $(W_0 - W_1)/W_0$) for the **PHEPGA1** coacervate was determined, where W_0 represents the initial weight of Nile red in the polymeric coacervate, and W_1 represents the remaining weight of the Nile red in the supernatant solution.

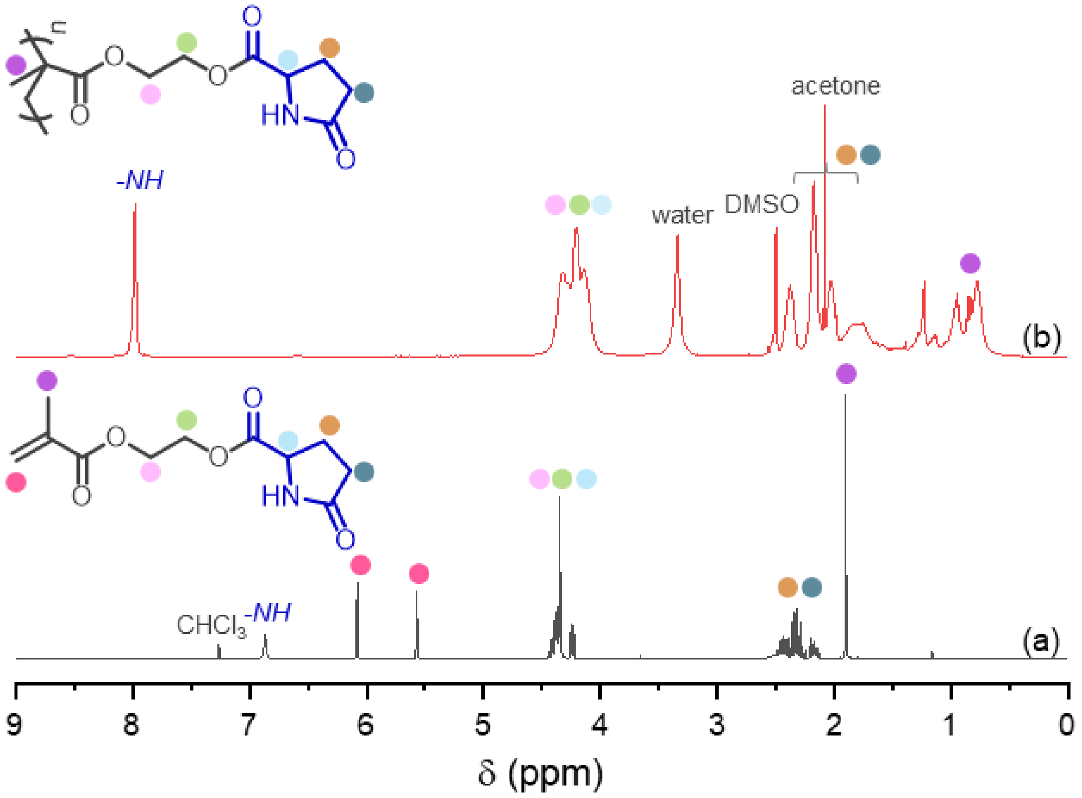

Fig. S1 ^{1}H NMR spectra of (a) HEPGA monomer in $CDCl_3$, and (b) **PHEPGA1** homopolymer in DMSO-d_6.

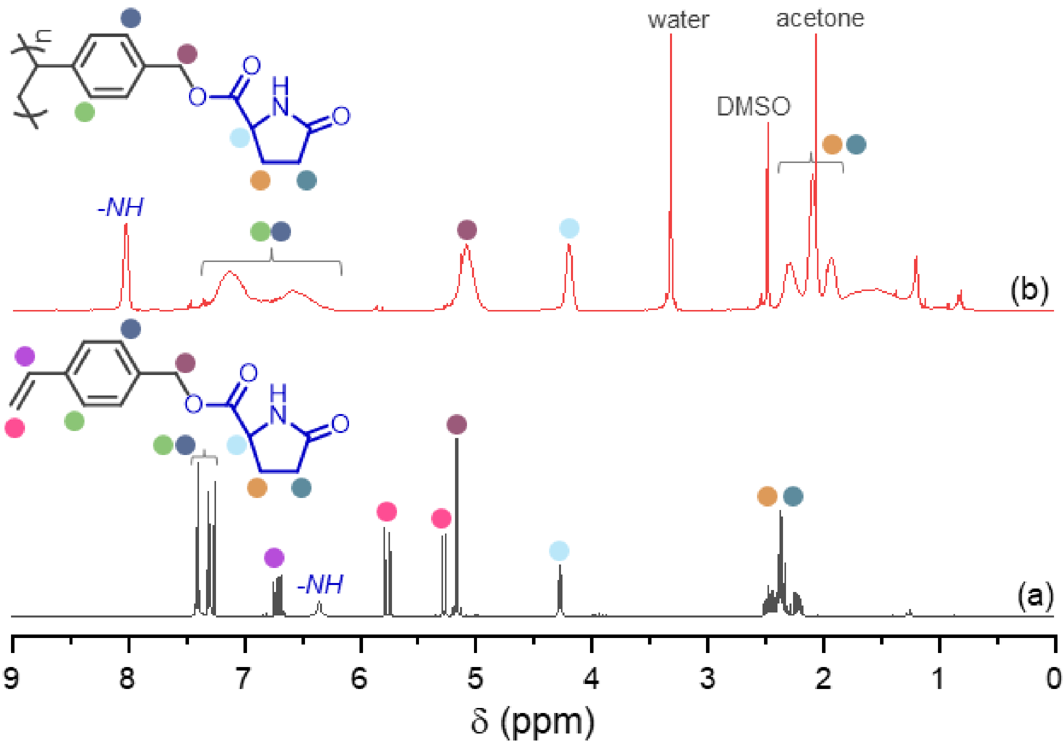

Fig. S2 ^{1}H NMR spectra of (a) VBPGA monomer in CDCl$_3$, and (b) **PVBPGA1** homopolymer in DMSO-d_6.

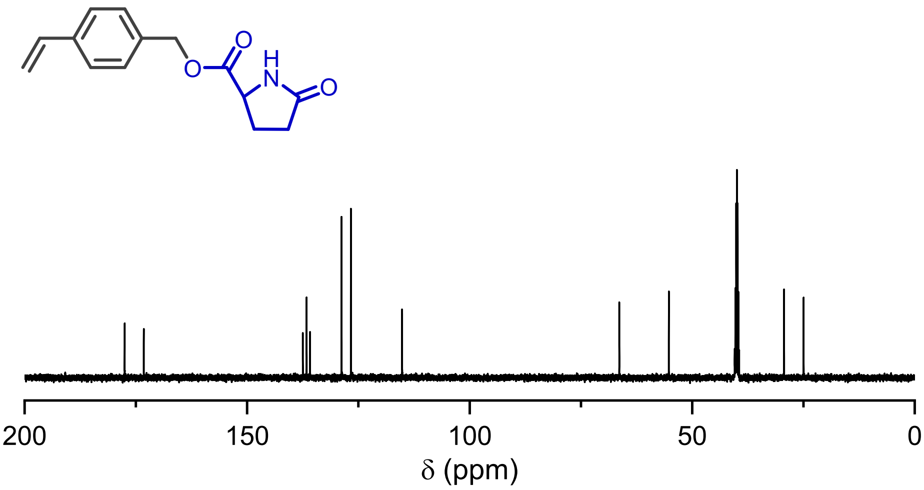

Fig. S3 ^{13}C NMR spectrum of VBPGA in CDCl$_3$.

Fig. S4 HRMS data of VBPGA. Calculated *m*/*z* for $[\mathrm{VBPGA + Na}]^+$ = 268.0944; observed = 268.0932.

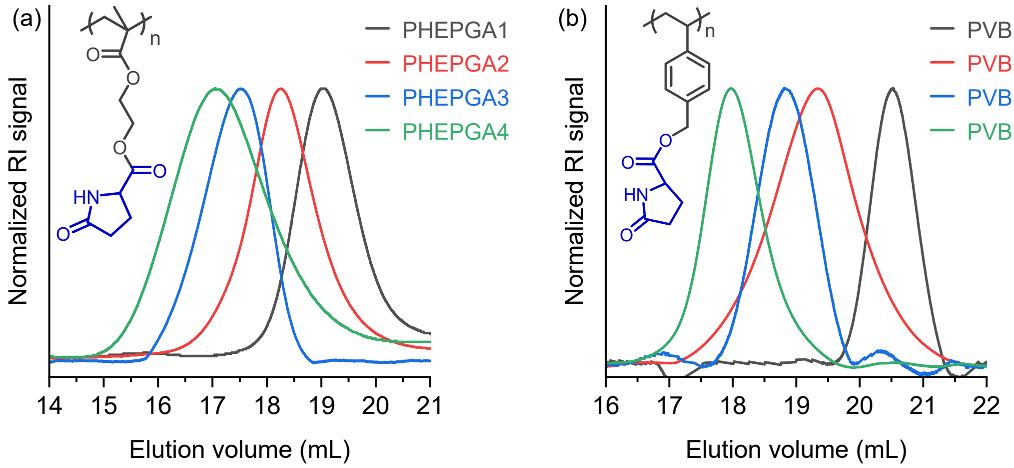

Fig. S5 SEC traces of (a) **PHEPGA** and (b) **PVBPGA** homopolymers of varying molecular weight.

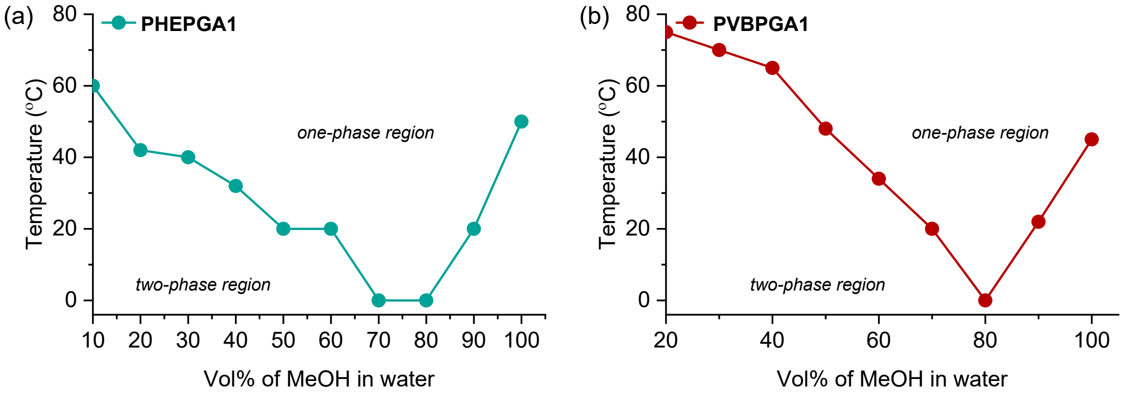

Fig. S6 Variation of cloud point temperatures of 0.2 wt% **PHEPGA1** (a) and 0.2 wt% **PVBPGA1** (b) at various contents of methanol (MeOH) in water.

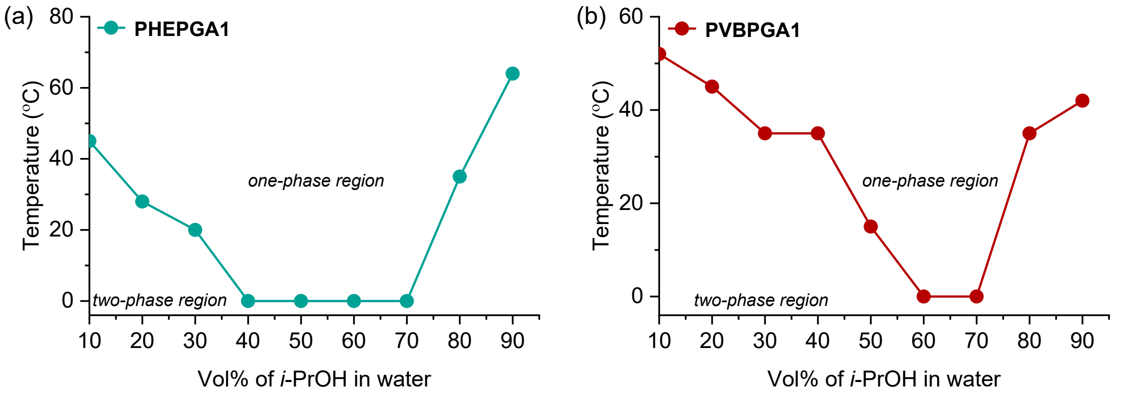

Fig. S7 Variation of cloud point temperatures of 0.2 wt% **PHEPGA1** (a) and 0.2 wt% **PVBPGA1** (b) at various content of isopropanol (*i*-PrOH) in water.

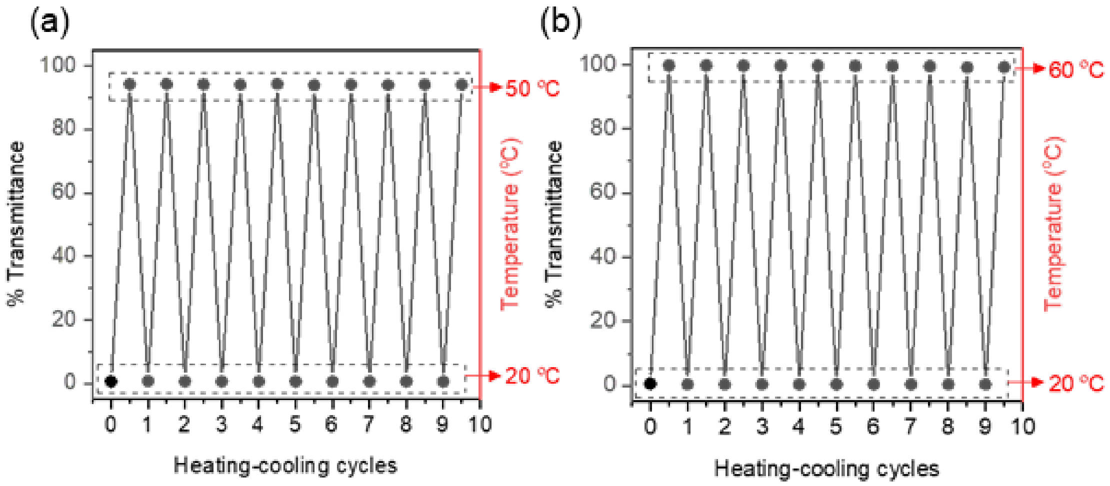

Fig. S8 Heating and cooling reversibility curves of the % transmittance for **PHEPGA1** in 20 % methanol-water (a), and **PVBPGA1** in 50% methanol-water (b).

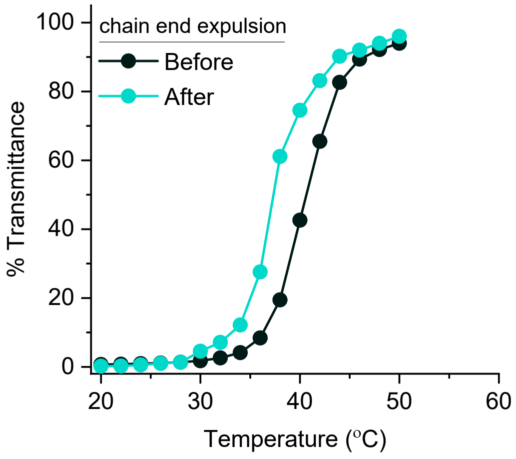

Fig. S9 Effect of chain end expulsion on the UCST behavior of **PHEPGA1** in 20% methanol-water.

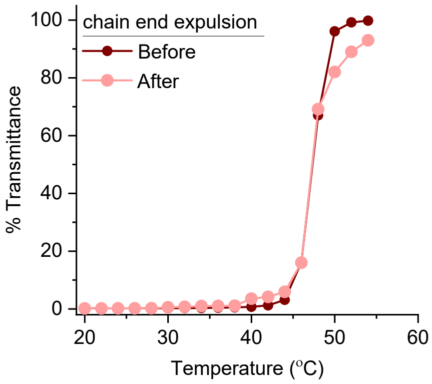

Fig. S10 Effect of chain end expulsion on the UCST behavior of **PVBPGA1** in 50% methanol-water.

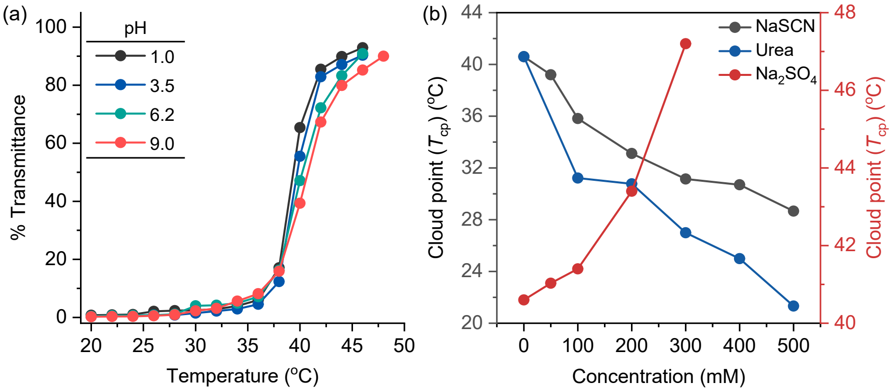

Fig. S11 (a) Effect of pH on the UCST behavior of **PHEPGA1** in 20% methanol-water. (b) Influence of kosmotropic salt, chaotropic salt, and agent (urea) on the UCST cloud point of **PHEPGA1** in 20% methanol-water.

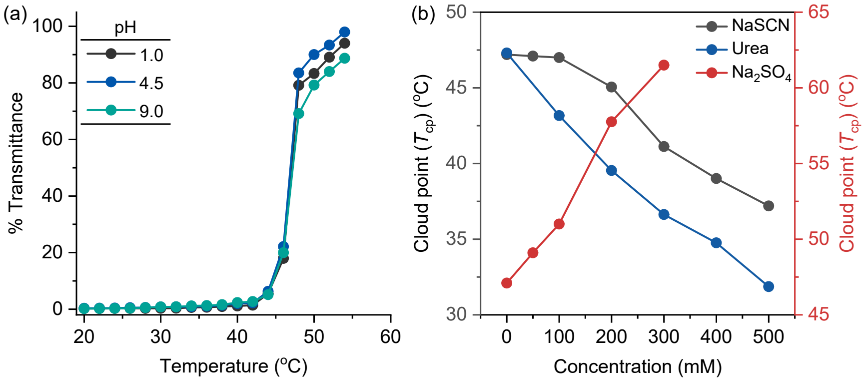

Fig. S12 (a) Effect of pH on the UCST phase transition of **PVBPGA1** in 50% methanol-water. (b) Influence of kosmotropic salt, chaotropic salt, and agent (urea) on the UCST cloud point of **PVBPGA1** in 50% methanol-water.

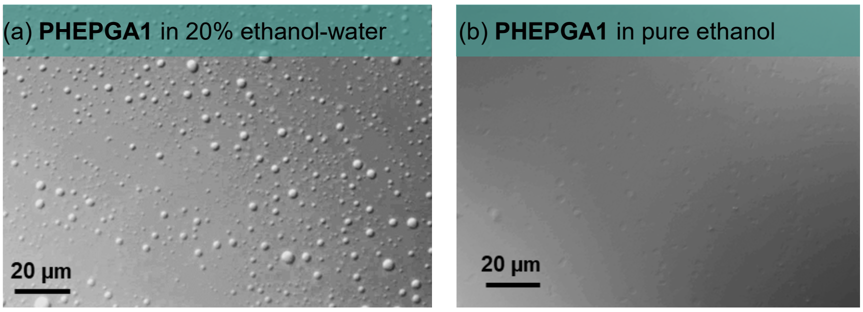

Fig. S13 Optical microscopic images of 1 wt% **PHEPGA1** solution (a) in 20% ethanol-water mixture and (b) in ethanol.

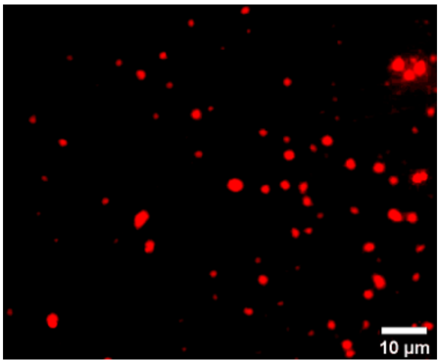

Fig. S14 Nile red encapsulated **PHEPGA1** coacervate prepared in 20% ethanol-water.

References

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