## **Supporting Information**

## Self-Healable Antifreeze Hydrogel Based on Dense Quadruple Hydrogen Bonding

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\*(S.C.) Telephone: +82-2-320-1686. Fax: +82-2-3143-1129. E-mail: shchoi@hongik.ac.kr \*(K.C.) Telephone: +82-2-880-7431. Fax: +82-2-873-1548. E-mail: khchar@snu.ac.kr **Synthesis**  $\alpha$ -methacryloyl- $\omega$ -(6-(3-(6-methyl-4-oxo-1,4-dihydropyrimidin-2of yl)ureido)hexylcarbamoyloxy)poly(ethylene glycol) (PEGMA-UPy). PEGMA-UPy was obtained by 2(6-isocyanatohexylaminocarbonylamino)-6-methylthe reaction of 4[1H]pyrimidinone (UPy-NCO) and hydroxyl-terminated PEG methacrylate (PEGMA-OH) as illustrated in Figure S1(a). UPy-NCO was synthesized by reacting 2-amino-4-hydroxy-6methylpyrimidine (20 g, 0.16 mol) and hexamethylene diisocyanate (200 ml, 1.25 mol) as reported previously.<sup>1</sup> Then, UPy-NCO (3.2 g) was reacted with PEG methacrylate with hydroxyl group (MW = 360 g/mol, 15 ml) in the presence of dibutyltin dilaurate in the 100 ml anhydrous chloroform (75 °C, reflux) for 12 hours. The solution was filtered off, and precipitated in *n*-hexane. Then, the crude product was re-dissolved in dichloromethane, filtered off, and precipitated again into the *n*-hexane. These steps were repeated at least two times to remove PEGMA-OH completely. After the purification steps, the crude product was obtained as a sticky white powder by drying in vacuum. The unreacted isocyanate in the crude product was removed by mixing with 10 g of silica at 65 °C for 1 h. The solution was filtered off and precipitated with *n*-hexane. The obtained white solid was dried in the vacuum oven. The final product (PEGMA-UPy) was obtained with 95% purity due to the residual *n*-hexane (4.0 g, 55%) yield) and characterized by <sup>1</sup>H-NMR (Advance III 400, Bruker), <sup>13</sup>C-NMR (Advance III 400, Bruker), and LC-TOF-MS (JMS-T100LP 4G, JEOL) as displayed in Figure S1(a), (b), and (c), respectively. Based on the integral of the proton of ethylene glycol units in the PEGMA-UPy and the LC-TOF-MS of PEGMA-UPy, we confirmed that the number-average molecular weight of PEGMA-UPy is 592 g/mol (containing 3~4 ethylene glycol units in a monomer). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 13.12 (s, 1H, CH<sub>3</sub>CN<u>H</u>), 11.85 (s, 1H, CH<sub>2</sub>NH(C=O)N<u>H</u>), 10.13 (s, 1H, CH<sub>2</sub>NH(C=O)NH), 6.12 (s, 1H, HCH=C(CH<sub>3</sub>)), 5.83 (s, 1H, CH=CCH<sub>3</sub>), 5.57 (s, 1H, HCH=C(CH<sub>3</sub>)), 5.02 (s, 1H, CH<sub>2</sub>-(C=O)NHCH<sub>2</sub>), 4.1 (m, 4H,  $HCH=C(CH_3)-$ 

(C=O)OC<u>H<sub>2</sub>CH<sub>2</sub>-), 3.66 (s, 12H, -CH<sub>2</sub>CH<sub>2</sub>O-), 3.23 (m, 2H, CH<sub>2</sub>-(C=O)NHC<u>H<sub>2</sub>), 3.15 (m, 2H, CH<sub>2</sub>-NH(C=O)NH(C<sub>5</sub>H<sub>5</sub>ON<sub>2</sub>)), 2.22 (s, 3H, CH=CC<u>H<sub>3</sub>), 1.94 (s, 3H, HCH=C(CH<sub>3</sub>)), 1.59 (m, 2H, NCH<sub>2</sub>C<u>H<sub>2</sub>), 1.49 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>-NH(C=O)NH(C<sub>5</sub>H<sub>5</sub>ON<sub>2</sub>)), 1.35 (m, 4H, CH<sub>2</sub>CH<sub>2</sub>C<u>H<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>). ). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  172.8, 167.1, 156.3, 154.4, 148.2, 135.9, 125.7, 106.3, 70.4, 69.4, 68.9, 67.6, 67.1, 66.7, 63.7, 62.8, 62.0, 40.5, 39.5, 29.6, 26.1, 25.4, 18.7, 18.1. LC-TOF MS: observed *m*/*z* = 424.273, 468.305, 512.337, 556.369, 600.402, 644.432, 688.464, 732.495, 776.528, 820.558, 864.591 ([M+H]<sup>+</sup>).</u></u></u></u></u>



**Figure S1.** (a) Synthetic scheme, (b) <sup>1</sup>H NMR spectra and (c) <sup>13</sup>C NMR spectra of PEGMA-UPy monomer.



Figure S2. LC-TOF-MS result of PEGMA-UPy monomer.

Synthesis of Px polymer. The amphiphilic random copolymers, poly(poly(ethylene glycol)) methacrylate-co-( $\alpha$ -methacryloyl- $\omega$ -(6-(3-(6-methyl-4-oxo-1,4methyl ether dihydropyrimidin-2-yl)ureido)hexylcarbamoyloxy)poly(ethylene glycol))) (P(PEGMA-co-(PEGMA-UPy)) with various mole fractions of UPy group were synthesized using reversible addition-fragmentation chain transfer (RAFT) polymerization, labelled Px where x indicates the mole fraction of UPy repeat unit. As a representative synthesis procedure, synthesis of P45 polymer is provided in detail. Poly(ethylene glycol) methyl ether methacrylate (PEGMA, 4.09 g, 8.18 mmol), PEGMA-UPy (4.50 g, 6.69 mmol), ECT (6.52 mg, 0.0248 mmol) and AIBN (0.61 mg, 0.0037 mmol) were dissolved in 21 ml of anhydrous N,N-dimethylformamide (DMF) under argon condition in a Schlenk flask, and followed by several freeze-pump-thaw processes. Then the polymerization was performed at 75 °C for 4 h, and then the polymer was recovered by precipitation in cold diethyl ether. For the complete removal of DMF and unreacted monomers, the polymer was redissolved in dichloromethane and precipitated again in cold diethyl ether at least three times.

**Calculation of Degree of Polymerization (DP)** The degree of polymerization was characterized by <sup>1</sup>H nuclear magnetic resonance spectroscopy (<sup>1</sup>H-NMR, Advance III 400, Bruker) as shown in Figure S3. DP was calculated as DP = conversion×[M]<sub>0</sub>/[CTA], where [M]<sub>0</sub> is the initial molar concentration of the monomer and [CTA] is the concentration of chain transfer agent, ECT. In the polymerization, a small amount of dibenzyl ether was added as an indicator, and the conversion was calculated by comparing the initial and final integral ratio of the unreacted monomer (s, 1H, 6.17 ppm) and the indicator (C<sub>6</sub>H<sub>5</sub>–CH<sub>2</sub>-O, s, 2H, 4.55 ppm). For instance, the conversion of P45 was calculated as

Conversion = 
$$1 - \frac{[M]}{[M]_0} = 1 - \frac{0.486}{1.305} = 0.628$$
 and DP = Conversion × 600  $\approx$  376,

where 0.486 is the initial ratio before the polymerization and 1.305 is the final ratio after the polymerization.



**Figure S3.** <sup>1</sup>H NMR spectra of the monomer conversion during the polymerization for P35, P40, P45 and P50 polymers.



**Figure S4.** Solubility comparison between reference monomer (REF 2, 3) and synthesized PEGMA-UPy monomer in 1,4-dioxane and *N*,*N*-dimethylformamide (DMF) at 25 °C. Compared to the reference molecule, PEGMA-UPy shows enhanced solubility up to 0.6 M in both 1,4-dioxane and DMF.

**SAXS Model.** The SAXS profiles were fit to the scattering model representing randomly distributed ellipsoidal aggregates interacting through the effective hard-sphere potential. This model considers the single-particle and the inter-particle scattering from the nano-sized prolate aggregates. According to earlier reports, the aggregates possess anisotropy with a long axis along the UPy packing,<sup>4, 5</sup> therefore, a prolate geometry was chosen to optimize the fitting quality. Since the polymer volume fractions of these hydrogels were more than 0.4, the density fluctuations of the hydrated polymer segments (which connects the prolate particles) in aqueous media are relatively negligible.

The prolate aggregates were assumed to have a spatially homogeneous scattering length density (SLD) and the uniform principal radii, { $R_{\min}$ ,  $R_{\min}$ ,  $R_{\max}$ }, where  $R_{\min}$  and  $R_{\max} = \varepsilon R_{\min}$  ( $\varepsilon > 1$ ) are the principal radii along the minor and major axes, respectively.<sup>6</sup> Hence, the model function for the scattering profile is

$$I(q) = cP(q)S(q) + B, \tag{S1}$$

where *q* is the scattering vector, *c* is a prefactor of arbitrary unit, P(q) is the form factor of single prolate aggregate, S(q) is the structure factor accounting for the interparticle interference, and *B* is the background intensity. The effective hard-sphere structure factor based on the Percus–Yevick closure was chosen for S(q), since the shell composed of oligo(ethylene glycol) linkers between UPy groups generates the excluded volume repulsive interaction between the prolate cores.<sup>7</sup> Hence, S(q) was parameterized with the effective hard-sphere radius and volume fraction,  $R_{\rm hs}$  and  $\varphi_{\rm hs}$ , respectively. It is noted that  $R_{\rm hs}$  is found to be larger than both  $R_{\rm min}$  and  $R_{\rm mai}$ , owing to the presence of the shell of oligo(ethylene glycol) side-chains.

P(q) was calculated by averaging the angular form factor over all possible orientation of the prolate after Kline,<sup>8</sup>

$$P(q, R_c) = \int_0^{\pi/2} F^2(q, \alpha) \sin \alpha \ d\alpha,$$
(S2)
S9

where the randomized orientation of the prolate particles was assumed, and  $\alpha$  is the angle between the long axis and the scattering vector q.  $F(q, \alpha)$ , the angle-dependent scattering amplitude of an ellipsoid, is given as

$$F(q, \alpha) = V_c(\rho_c - \rho_s)\Phi(qr_c) \quad . \tag{S3}$$

In eq S3,  $V_c = (4\pi/3)\varepsilon R_{\min}^3$  is the volume of the core,  $\Phi(x) = 3x^{-3}(\sin x - x \cos x)$ , and  $\rho_c$ and  $\rho_s$  are the scattering length densities of the core and the solvent medium, respectively, and  $r_c = R_{\min}(\sin^2 \alpha + \varepsilon^2 \cos^2 \alpha)^{1/2}$  is the orientation-dependent radii of the core and the entire aggregate. Lastly, the incoherent scattering and the errors in the background subtraction were aggregated into the term *B*. Hence, the fitting routine was performed using 6 fitting parameters (*c*,  $R_{\min}$ ,  $\varepsilon$ ,  $R_{hs}$ ,  $\varphi_{hs}$ , and *B*) to adjust the SAXS profile over the *q* range from 0.009 Å<sup>-1</sup> to 0.7 Å<sup>-1</sup>.

The fitting results are summarized in Table S2. As the molar fraction of UPy increases, the anisotropy ( $\varepsilon$ ) of UPy aggregates also increases. This is consistent with the previous observations, which reported the change of morphology from spherical to worm-like micelles as the UPy fractions in the hydrogel increased.<sup>4, 5</sup> However,  $R_{\min}$  remains almost independent on the UPy fraction, suggesting that the thickness of core is regulated by the full length of UPy group. The aggregation number of UPy groups, defined as the average number of groups composing a prolate core, was estimated by dividing the prolate volume by the molecular volume of the UPy group.

**Estimation of Gap Distance of Confinement.** To address the confinement effect of densely distributed UPy domains in associative physical hydrogels, the average gap distance needs to be estimated from the structural features. Assuming that the spatial distribution of prolate particles is random in orientation, it is convenient to use the particle number density to define

the gap distance of the confinement. For the hydrogel composed of UPy cores, the particle number density is defined as;

Particle Number Density 
$$= N_p/V = 1/v_{cell}$$
, (S4)

where *V* is the total volume of the hydrogel,  $N_p$  is the number of particles, and  $v_{cell}$  is the effective volume of the unit cells occupied by the UPy moieties and the oligo(ethylene glycol) side-chains. Furthermore, the total volume of UPy domains can be expressed by the following equation;

$$N_{p}v_{p} = f_{UPy}(\varphi_{poly}V) , v_{p} = \frac{4}{3}\pi R_{major}R_{minor}^{2} , f_{UPy} = \frac{xV_{UPy}}{xV_{PEG-UPy} + (1-x)V_{non-UPy}}$$
(S5)

where the  $v_p$  is the volume of the prolate UPy core obtained from the SAXS fitting results, which consist of the UPy and urethane moieties.  $\varphi_{poly}$  is the volume fraction of polymer and  $f_{UPy}$  is the volume fraction of associative moieties (i.e., UPy) in Px polymer.  $V_{UPy}$ ,  $V_{PEG-UPy}$  and  $V_{non-UPy}$  are the molar volume of UPy, PEGMA-UPy and PEGMA, respectively. Combining the equations above and assuming that the unit cell is radially expanded to a half of the gap distance ( $d_{gap}/2$ ) from the prolate surface, the effective volume of unit cell can be expressed as

$$v_{cell} = v_P / f_{UPy} \varphi_{poly} = \frac{4}{3} \pi \left( R_{major} + \frac{d_{gap}}{2} \right) \left( R_{minor} + \frac{d_{gap}}{2} \right)^2 .$$
(S6)

The characteristic gap distance of confinements was estimated by solving eq 6, and the results are summarized in Table S2.



**Figure S5.** Schematic representation of SAXS model fit based on the volume of unit cells occupied by the UPy moieties and PEG sidechains.



**Figure S6.** SAXS fitting results of the (a) P35 (b) P40 (c) P45 and (d) P50 containing 50 wt% of water at 25 °C.

**Table S1.** The structural parameters for the confinements of the P*x* hydrogels (x = 35-50 mol%) containing 50 wt% of water obtained from the molecular characteristics, the SAXS fitting, and the gap estimation.

UPy mole fraction, x	0.35	0.4	0.45	0.5
Polymer volume fraction in hydrogel, $\varphi_{pol}$ (%)	45	45	45	45
UPy volume fraction in total polymer, $f_{\rm UPy}$ (%)	18	21	23	25
Particle major axis, $R_{maj}$ (nm)	2.0	2.1	2.8	3.1
Particle minor axis, $R_{\min}$ (nm)	1.2	1.3	1.3	1.4
Particle volume, $v_p (nm^3)$	13	15	19	24
UPy aggregation number	35	41	53	66
Unit cell volume, $v_{cell} (nm^3)$	158	163	188	211
Gap distance, $d_{gap}$ (nm)	3.8	3.7	3.7	3.7

**Table S2.** The characteristic gap distance of the confinements in the P45 hydrogel with the various polymer concentration.

Polymer concentration (wt%)	40	45	50	55
Gap distance, $d_{gap}$ (nm)	4.3	3.9	3.7	3.4



**Figure S7.** DSC thermograms of the chemically crosslinked 50 wt% P(PEGMA) hydrogel upon cooling and heating. The upward indicates the exothermic direction.



**Figure S8.** Variable temperature small angle X-ray scattering (SAXS) profiles for P45 hydrogels (50 wt% water) upon cooling from 20 °C to -50 °C with the cooling rate of (a) 10 °C/min (b) 5 °C/min and (c) 1 °C/min. The SAXS profiles were vertically shifted for clarity. (d) Domain distance (*d*) change of the P45 hydrogel with the temperature decrease. The water content in the hydrogels is 50 wt%.

**Field emission scanning electron microscopy (FE-SEM).** FE-SEM (JSM-7800F Prime, JEOL) was conducted to monitor the formation of ice crystalline domains upon cooling. Three different freezing procedures were applied for the freezing of P45 hydrogels (50 wt% water); (1) quenching from room temperature into the liquid nitrogen, (2) fast cooling to -60 °C in a temperature-controlled refrigerator for 1 hours, and followed by quenching into the liquid nitrogen, and (3) slow cooling with a cooling rate of 1 °C/min down to -60 °C, and followed by quenching into the liquid nitrogen. All frozen hydrogels were freeze-dried for 48 hours (OPERON, Korea) to remove water molecules. The cross-sections of freeze-dried hydrogels were obtained by the cryo-fracture in the liquid nitrogen, and the images of P45 hydrogels were obtained as shown in Figure S9.



**Figure S9.** FE-SEM images for freeze-dried P45 hydrogel prepared by different processes; (a) slow cooling from the room temperature to -60 °C, and followed by quenching into the liquid nitrogen. (b) rapid cooling from room temperature to the -60 °C, and followed by the quenching into the liquid nitrogen. (c) Rapid quenching from room temperature into the liquid nitrogen.



**Figure S10.** (a) Frequency-dependent storage (filled) and loss (open) moduli ( $\gamma = 1\%$ ) for 50 wt% P45 hydrogels measured at various temperature. (b) Time-temperature superimposed loss factor (tan  $\delta$ ) for 50 wt% P45 hydrogels.



**Figure S11.** Time-temperature superposed master curve and the Arrhenius relationship between the horizontal shift factor ( $a_T$ ) and the inverse temperature (1000/T) for (a) P35 (b) P40, and (c) P50 hydrogels containing 50 wt% water at the reference temperature of 25 °C ( $\gamma = 1\%$ ).



Figure S12. Linear relationship between the activation energy of junctions obtained from horizontal shift factor  $(a_T)$  and the mole fractions of ureidopyrimidinone in the gelators. The water content in the hydrogels was controlled to 50 wt%.



**Figure S13.** Strain sweep test for P45 hydrogel (T = 20 °C,  $\omega$  = 5 rad/s).

**X-ray Diffraction.** For the estimation of mean ice crystal size, the XRD peak were fitted by sum of Gaussian distribution function with equation S7,

$$I(2\theta) = \mathbf{B} + \sum I_{0,i} exp(-\frac{(2\theta - 2\theta_0)^2}{2\sigma^2})$$
(S7)

and the full with half maximum (FWHM) was obtained as FWHM =  $2.3548\sigma$ . From the obtained FWHM for the crystal plane, the size of ice crystal was estimated by Scherrer equation,

$$\tau = \frac{K\lambda}{\sqrt{(FWHM)^2 - B_0^2 \cos\theta}}$$
(S8)

where  $B_0$  was obtained as  $0.07^{\circ}$  (1.222 ·10<sup>-3</sup> rad) from the instrumental broadening and the shape factor K is assumed to be 0.9 and the wavelength of X-ray ( $\lambda$ ) was 0.15418 nm.



**Figure S14.** (a) XRD data of P40 and P45 hydrogels containing 45 wt% gelators. (b) XRD data of P35, P40 and P45 hydrogels containing 55 wt% gelators. The dashed black lines indicate the Bragg reflections from the hexagonal ice, black triangles represent the reflections from the cubic ice, the brown triangles reveal the reflection from the substrate (sample holder) and the open triangle represents the short-range ice with less crystallinity.

Sample code (10) Hexag	(100)	(002)	(111)	Short
	Hexagonal	Hexagonal	Cubic	Range Ice
P45_50 wt%60 °C	3.1 nm	(-)	10.9 nm	1.9 nm
P45_55 wt%60 °C	(-)	(-)	(-)	1.5 nm

**Table S3**. Mean crystalline size of ice estimated by Scherrer equation.

## REFERENCES

(1) Folmer, B. J.; Sijbesma, R.; Versteegen, R.; Van der Rijt, J.; Meijer, E., Supramolecular polymer materials: Chain extension of telechelic polymers using a reactive hydrogen-bonding synthon. *Adv. Mater.* **2000**, *12*, 874-878.

(2) Zhang, G.; Chen, Y.; Deng, Y.; Ngai, T.; Wang, C., Dynamic Supramolecular Hydrogels: Regulating Hydrogel Properties through Self-Complementary Quadruple Hydrogen Bonds and Thermo-Switch. *ACS Macro Letters* **2017**, *6*, 641-646.

(3) Choi, J.; Yoon, J.; Ahn, K. H.; Choi, S.-H.; Char, K., Injectable hydrogels with improved mechanical property based on electrostatic associations. *Colloid Polym. Sci.* **2020**, 1-10.

(4) Dankers, P. Y.; Hermans, T. M.; Baughman, T. W.; Kamikawa, Y.; Kieltyka, R. E.; Bastings, M. M.; Janssen, H. M.; Sommerdijk, N. A.; Larsen, A.; van Luyn, M. J.; Bosman, A. W.; Popa, E. R.; Fytas, G.; Meijer, E. W., Hierarchical formation of supramolecular transient networks in water: a modular injectable delivery system. *Adv. Mater.* **2012**, *24*, 2703-2709.

(5) Goor, O.; Hendrikse, S. I. S.; Dankers, P. Y. W.; Meijer, E. W., From supramolecular polymers to multi-component biomaterials. *Chem. Soc. Rev.* **2017**, *46*, 6621-6637.

(6) Pedersen, J. S., Form factors of block copolymer micelles with spherical, ellipsoidal and cylindrical cores. *J. Appl. Crystallogr.* **2000**, *33*, 637-640.

(7) Percus, J. K.; Yevick, G. J., Analysis of classical statistical mechanics by means of collective coordinates. *Phys. Rev.* **1958**, *110*, 1.

(8) Kline, S. R., Reduction and analysis of SANS and USANS data using IGOR Pro. *J. Appl. Crystallogr.* **2006**, *39*, 895-900.