Supporting Information for

Nucleobase-Grafted Polycaprolactones as Reversible Networks in a Novel Biocompatible Material

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Experiment Section.

Materials. ε-Caprolactone (εCL, 99.5 %, ACROS), dichloromethane (DCM, 99 % ACROS), and dimethyl formamide (DMF, 99 %, Fisher Chemical) were dried over calcium hydride (CaH_2, 95 %, ACROS) for 24 h and then distilled under reduced pressure prior to use. Uracil (U, 99 %, ACROS), adenine (A, 99 %, Sigma), glacial acetic acid (HPLC grade, TEDIA), tetrahydrofuran (THF, HPLC

grade, TEDIA), acetonitrile (ACN, anhydrous, Aldrich), ethyl acetate (EtOAc, ACS reagent, Sigma-Aldrich), hexane (HPLC grade, Sigma-Aldrich), sodium azide (SHOWA, 98 %), propargyl bromide (Fluka, 80 % in Toluene), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, ACROS, 98 %), methanol (MeOH, HPLC grade, TEDIA), 1,1'-carbonyldiimidazole (CI, 97 %, ACROS), propargyl alcohol (PA, 99 %, ACROS), *N*,*N*,*N*′,*N*″,*N*″-Pentamethyldiethylenetriamine (PMDETA, 99 %, Aldrich), and 3-Chloroperbenzoic acid (*m*-CPBA, 77 %, Aldrich), benzyl alcohol (99 %, ACROS), 2-Chlorocyclohexane (98 %, ACROS), and Sodium hydrosulfite ($Na_2S_2O_4$, 85 %, SHOWA) were all used as received. Copper(I) bromide (CuBr) was stirred in glacial acetic acid overnight, filtered, and then rinsed with absolute ethanol under a blanket of argon and dried under vacuum at 60 °C overnight. Tin(II) 2-ethylhexanoate (~ 95%) was purchased from Sigma-Aldrich and azeotropically distilled from toluene three times prior to use. Nucleobase-functioned monomers 9-(2'-hydroxyethyl) adenine [HEA] were synthesized from adenine and ethylene carbonate according previous studies.[1] Propargyl ester of carbonyl imidazole [PPA-CI] was synthesized from 1,1'-carbonyldiimidazole and propargyl alcohol according a procedure described in the literature.[2] Linear PCL was obtained by ring-opening polymerization at previously described and it has a quoted molecular weight of M_n = 22530.[3]

Characterizations. Analytical TLC was performed on commercial Merck Plates coated with silica gel GF254 (0.24 mm thick). Silica gel for flash chromatography was from Merck Kieselgel 60 (230-400 mesh, ASTM). FT-IR spectra were recorded using a Nicolet Avatar 320 FTIR Spectrometer; 32 scans were collected at a spectral resolution of 1 cm^{-1}. The conventional KBr disk method was employed: the sample was dissolved in DMF, then cast onto a KBr disk, and dried under vacuum at 120 °C. ^1H NMR spectra were recorded in $CDCl_3$ and d_6-DMSO solution using a Varian Inova 500 MHz spectrometer equipped with a 9.395 T Bruker magnet and operated at 500 MHz. The weight-average molecular weight (M_w), number-average molecular weight (M_n), and PDI (M_w/M_n) were measured using a Waters 410 GPC system equipped with a refractive index detector and three Ultrastyragel columns (100, 500,

and 1000 Å) connected in series. DMF was used as solvent and the system was operated at 25 °C and calibrated using polystyrene (PS) standards. The molecular weight determined from polystyrene standards is not valid in such cases, it serves only for comparison. Thermal analysis was carried out using a DSC instrument (TA Instruments Q-20) under an atmosphere of dry N_2. Samples were weighed (3-5 mg) and sealed in an aluminum pan, which was scanned from -90 to 160 °C at a scan rate of 20 °C/min. WAXD spectra of powders were obtained using a Rigaku D/max-2500 X-ray diffractometer. The radiation source was Ni-filtered Cu Kα radiation at a wavelength of 0.154 nm. The voltage and current were set at 30 kV and 20 Ma, respectively. Bragg's law ($\lambda = 2d\sin\theta$) was used to compute the *d*-spacing corresponding to the complementary behavior. DLS measurements were performed in DMF solution using a Malvern Zetasizer Nano S90 at a wavelength of 532 nm, at angular range of 90 ° and temperature at 25 °C. All samples were filtered before DLS measurement using a 0.5 μm filter. The measured intensity correlation functions were subjected to CONTIN analysis. Apparent hydrodynamic diameters of aggregations were calculated according to the Stokes-Einstein equation. The cumulant and CONTIN methods are valid for rigid polymers. TEM images were obtained using a JEOL TEM-1200EX II instruments operated at 120 KV. AFM micrographs were recorded at 25 °C in air using a Digital Instrument Multimode Nanoscope IV operating in the tapping regime mode using silicon cantilever tips (PPP-NCH-50, 204-497 kHz, 10-130 N/m).

Cell Proliferation. In vitro cytotoxicity was evaluated using NCTU clone L929 mouse fibroblasts purchased from Japanese Collection of Research Bioresources (JCRB). The fibroblasts were cultured in BD-Falcon tissue culture well plate in modified Eagle's minimal essential medium supplemented with 10 % fetal bovine serum. The cells were incubated at 37 °C in a 5 % CO_2 atmosphere, and the growth medium was replaced with fresh one once every 24 h. In addition, Samples were dispersed with a 100 μL of Dulbecco's modified Eagle's medium solution (DMEM, Sigma Chemical Company, USA), and mixed with 20 μL of trypan blue to count the number of cells using a hemocytometer. The status of

adhesion and growth of the cells were observed after 24 h by a digital camera.

Blend Preparation. Blends of the copolymers poly(εCL-*g*-Uracil) and poly(εCL-*g*-Adenine) were prepared through solution blending. DMF solutions containing 5 wt % of the polymer mixture were stirred for 6-8 h; the solvent was then left to evaporate slowly at room temperature for 24 h. The resulting blend films were dried at 60 °C for 2 days.

Synthesis of N^1-(Prop-2-yne-1-yl)uracil This compound was prepared according to the literature procedure.[4] (yield = 78 %). ^{1}H NMR (500 MHz, DMSO, δ): 11.35 (br s, 1H, –N*H*–), 7.68 (d, *J* = 6.0 Hz, 1H, –N–C*H*–), 5.61 (d, *J* = 6.0 Hz, 1H, –CO–C*H*–), 4.49 (d, *J* = 3.0 Hz, 2H, –CH_2–), 3.37 (t, *J* = 3.0 Hz, 1H, –C≡C*H*).

Synthesis of Propargyl Ester of Adenine 9-(2'-hydroxyethyl) adenine ([HEA], 1 g, 5.58 mmol) and 20 mL DMF were loaded into a dry round-bottom flask. Then, propargyl ester of carbonyl imidazole ([PPA-CI], 1.25 g, 8.34 mmol) was quickly added. The solution was stirred at 60 °C in an oil bath for 12 h. DMF was then removed by evaporation under reduced pressure to obtain a white solid. Finally, the product was purified by recrystallization from dichloromethane. Yield: 1.2 g (54 %). ^{1}H NMR (500 MHz, DMSO, δ): 8.10 (2 H, s, Ar*H*), 7.21 (1 H, s, NH_2), 4.67 (s, 1H, −C=O−CH_2−), 4.47 (2 H, t, CH_2), 4.43 (2 H, t, CH_2), 3.58 (t, 1H, –C≡C*H*). ^{1}H NMR (500 MHz, DMSO, δ): 156.9 (−O*C*=OO−), 154.4 (−N=*C*−), 153.4 (−N=*C*−), 150.6 (−N−*C*−N−), 141.8 (−N=*C*−N−), 119.4 (−C−*C*−N−), 79.3 (−CH≡*C*−), 78.4 (−*C*H≡C−), 66.7 (−CO*C*H_2−), 56.0 (−CH≡C−*C*H_2−), 42.79(−N−*C*H_2−).

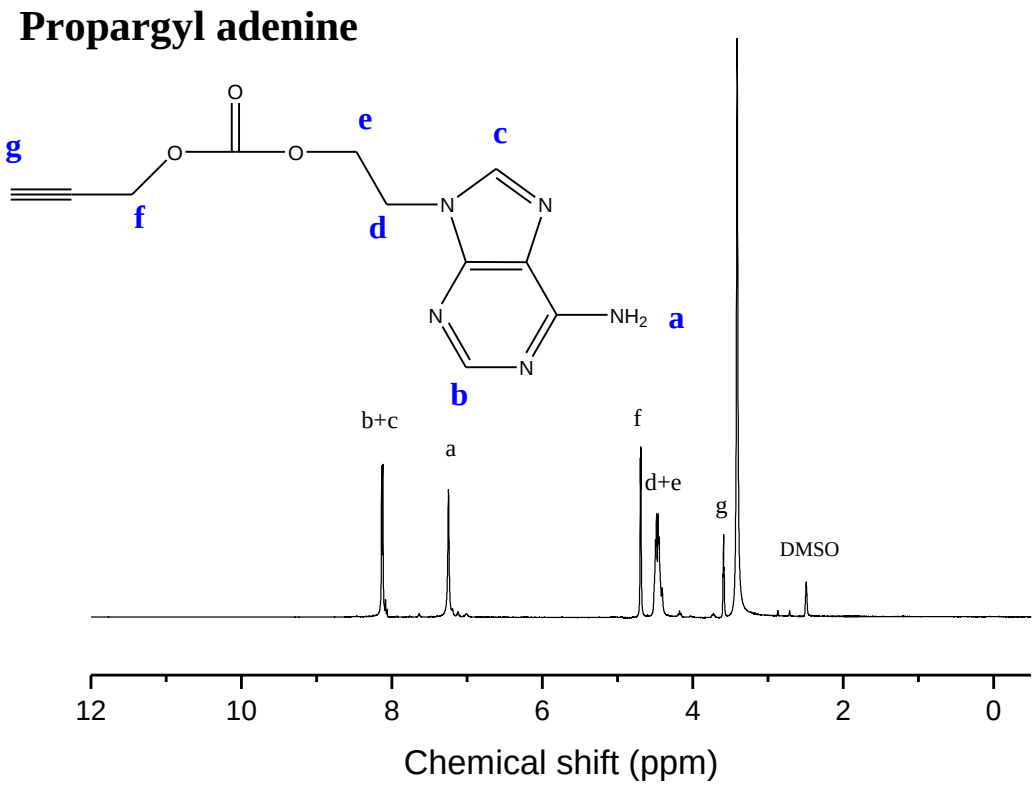

Figure S1. ^{1}H NMR spectra of Propargyl ester of adenine.

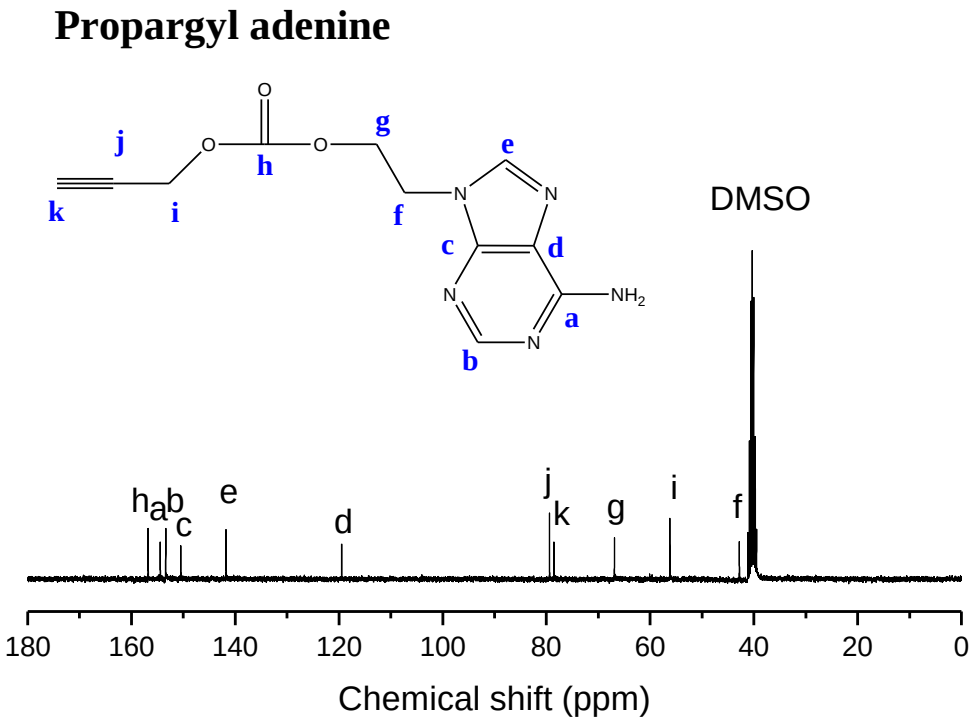

Figure S2. ^{13}C NMR spectra of Propargyl ester of adenine.

Synthesis of *α*-Chloro-*ε*-caprolactone[5] 2-chlorocycloxehanone (10 g, 75 mmol) and *m*-CPBA (20 g, 81 mmol) were dissolved in 300 mL dichloromethane and stirred for 48 h at 25 °C. The reaction flask was then cooled to 0 °C for precipitation of *m*-chlorobenzoic acid. The solution was filtered and washed in succession with $Na_2S_2O_4$ and saturated $NaHCO_3$ aqueous solution for three times, respectively. After being dried over $MgSO_4$, the organic phase was concentrated under reduced pressure. The residue was distilled under reduced pressure, the *α*-chloro-*ε*-caprolactone (bp = 75–77 °C at 0.1 mmHg) was collected. Yield: 84 %. ^{1}H NMR (500 MHz, $CDCl_3$, δ): 4.6–4.75 (1H, m, –C*H*ClCO–), 4.20–4.40 (1H, m, –CH_2O–), 1.60–2.10 (6H, m, –CH_2CH_2CH_2–).

Synthesis of Poly(αCl*ε*CL-*co*-*ε*CL) Copolymer Random copolymerization was carried out at 120 °C in toluene in a dried glass reactor. 3 g (20 mmol) of *α*-chloro-*ε*-caprolactone, 7 g (61 mmol) *ε*-caprolactone, 20 mL toluene, $Sn(Oct)_2$ (0.743 g, 1.83 mmol), and 23 µL benzyl alcohol (1 mmol) were sequentially added to the reactor through a rubber septum with a syringe. After 24 h of polymerization, an excess acetic acid (0.2 mL acetic acid/0.8 ml toluene) was added, and the copolymer was recovered by precipitation in cold methanol. ^{1}H NMR (500 MHz, $CDCl_3$, δ): 4.2–4.3 (poly(*α*Cl*ε*CL-*co*-*ε*CL), 1H per repeating unit, –C=O–Cl*H*–), 4.1–4.2 (poly(*α*Cl*ε*CL-*co*-*ε*CL), 2H per repeating unit, –COO–CH_2–), 3.9–4.1 (polycaprolactone, 2H per repeating unit, –COOCH_2–), 2.1–2.4 (polycaprolactone, 2H per repeating unit, –CO=CH_2CH_2–), 1.8–2.0 (poly(*α*Cl*ε*CL-*co*-*ε*CL), 2H per repeating unit, –CClH–CH_2–), 1.2–1.6 (polycaprolactone and poly(αCl*ε*CL-*co*-*ε*CL), 10 H per repeating unit, –COCH_2CH_2CH_2CH_2– and –COCH_2CH_2CH_2–).

Synthesis of the Poly(*α*Az*ε*CL-*co*-*ε*CL) Copolymer Poly(*α*Cl*ε*CL-*co*-*ε*CL (2g, 0.067 mmol) was dissolved in 10 mL of DMF, and then NaN_3 (0.044g, 0.67 mmol) was added to the solution. The resulting solution was allowed to stir at 60 °C overnight and precipitated in excess methanol. After filtration, the polymer was dried for 24 h in a vacuum oven at 25 °C (yield = 83.5 %). ^{1}H NMR (500 MHz, $CDCl_3$, δ): 4.1–4.2 (poly(*α*Cl*ε*CL-*co*-*ε*CL), 2H per repeating unit, –COO–CH_2–), 3.9–4.1 (polycaprolactone, 2H per repeating unit, –COOCH_2–), 3.7–3.8 (poly(*α*Az*ε*CL-*co*-*ε*CL), 2H per

repeating unit, –COC*H*–CH_2–), 2.1–2.4 (polycaprolactone, 2H per repeating unit, –CO=C$H_2$$CH_2$–), 1.7–1.8 (poly(*α*Az*ε*CL-*co*-*ε*CL), 2H per repeating unit, –CNH–CH_2–), 1.2–1.6 (polycaprolactone and poly(αAzεCL-*co*-εCL), 10 H per repeating unit, –$COCH_2$CH_2CH_2CH_2– and –$COCH_2$CH_2CH_2–).

Synthesis of Poly(*ε*CL-*g*-Uracil) Poly(*α*Az*ε*CL-*co*-*ε*CL) (3 g, 0.25 mmol), N^1-(Prop-2-yne-1-yl)uracil (0.94 g, 6.3 mmol), and 50 μL PDMETA were dissolved in 30 mL of DMF in a dried glass reactor. Then, the reactor was subjected to two freeze-pump-thaw cycles, N_2 was introduced into the reactor when the mixture was frozen, and Cu(I)Br (30 mg) was quickly added under N_2 atmosphere. Finally the reactor was stirred at 60 °C for 24 h. The mixture was diluted with DMF and passed through a short column of neutral aluminum oxide. The solution was distilled under reduced pressure and purified by dialysis in DMF to completely remove the excess N^1-(Prop-2-yne-1-yl)uracil. Drying under vacuum gave pure nucleobase-grafted polycaprolactone. Yield: 71 %. ^{1}H NMR (500 MHz, DMSO, δ): 11.2–11.4 (1H, –CO–N*H*–CO–), 8.0–8.2 (1H, –N–C*H*–), 7.56–7.8 (1H, –N–C*H*–), 5.5–5.7 (1H, –CO–C*H*–). 5.0–5.1 (–NCH_2–).

Poly(*ε*CL-*g*-Adenine) was synthesis by a method similar to that for poly(*ε*CL-*g*-Uracil). Poly(*α*Az*ε*CL-*co*-*ε*CL) (3 g, 0.25 mmol) and propargyl ester of adenine (1.2 g, 50 mmol) were used. Yield: 69 %. ^{1}H NMR (500 MHz, DMSO, δ): 8.1–8.2 (2H, –*H*C=C–), 8.0–8.1 (2 H, Ar*H*), 7.4–7.5 (2 H, s, NH_2), 5.4–5.5 (1 H, –CO–CN*H*–), 5.1–5.2 (2 H, –NCH_2–), 4.1–4.2 (2H, t, –N–CH_2–), 3.3–3.4 (2H, t, –C=O–O–CH_2–). By using the similar method, we prepared poly(*ε*CL-*g*-Uracil/Adenine) from Poly(*α*Az*ε*CL-*co*-*ε*CL), N^1-(Prop-2-yne-1-yl)uracil, and adenine at 50/50 feed molar ratio ([Uracil/Adenine]).

The kinetic study of Poly(αCl*ε*CL-co-*ε*CL) Copolymer The kinetic study of tin catalyst mediated ROP ([*α*Cl*ε*CL]/[*ε*CL]=32/68) is shown in Figure S3 by monitoring the GPC traces of *α*Cl*ε*CL, *ε*CL and molecular weight evolution of copolymers. Indeed, it clearly shows that the monomer signal intensity both decreases and copolymer molecular weight increases simultaneously. We have made further discussion between *ε*CL and its chloride derivative really random. Figure S4 illustrates the ^{13}C NMR in carbonyl region of the random copolymer. Signals of the carbonyl atoms are known to be particularly

sensitive to sequence effects. Peaks a and b correspond to the homotriads C-C-C and ClC-ClC-ClC, where C represents εCL and αClεCL. The additional peaks, a', a", b', and b", in the carbonyl region refer to the heterotriads and confirm the randomness of the copolymer. In addition, four peaks are observed, indicative for diads. Peaks c and d correspond to the C-C and ClC-ClC homodiads, and the peaks c' and d' are involved in ClC-C heterodiads. These data indicate that the copolymerization between εCL and αClεCL are random.[6]

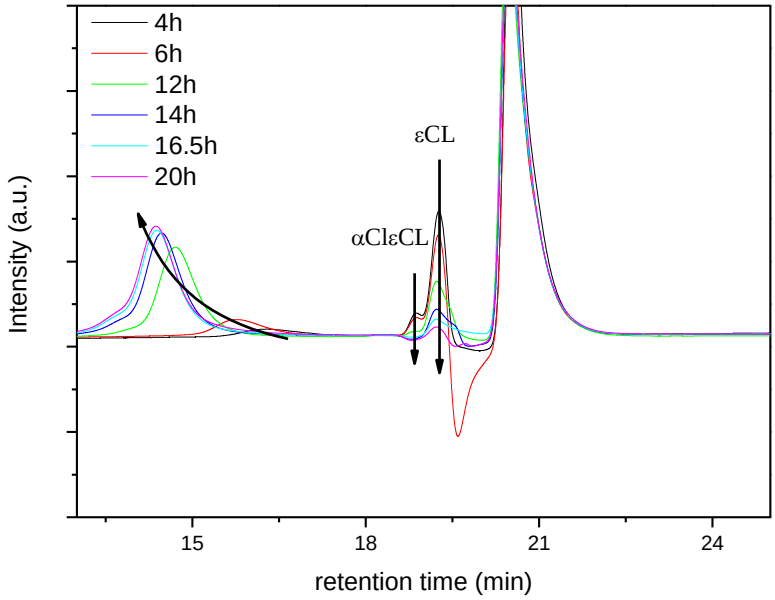

Figure S3. GPC traces of monomer αClεCL, monomer εCL, and Poly(αClεCL-*co*-εCL).

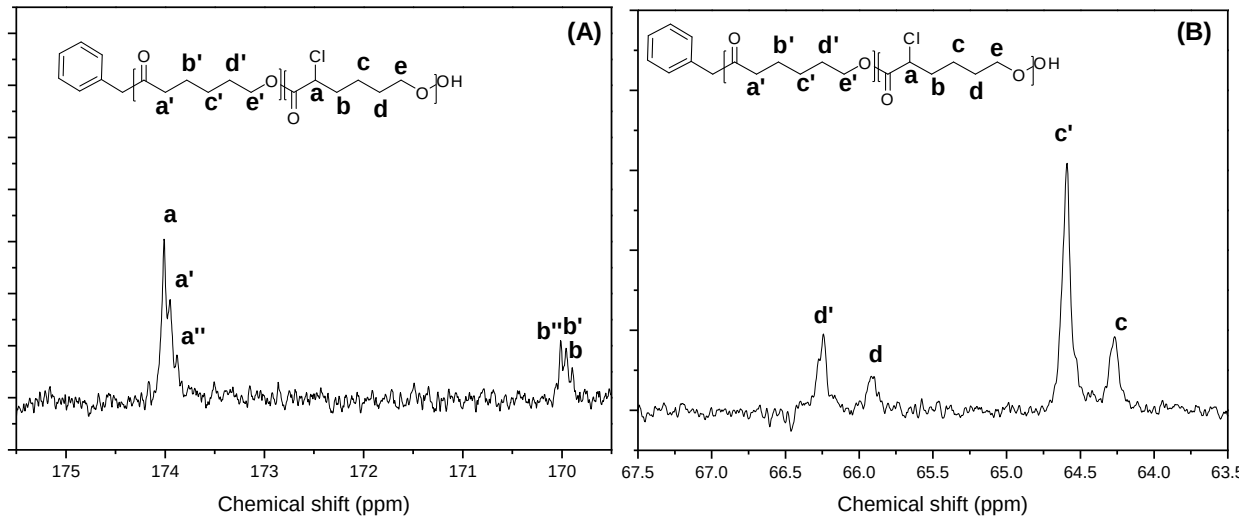

Figure S4. (A) ^{13}C NMR spectrum in the carbonyl region of Cl30-PCL (B) ^{13}C NMR spectrum in the -OCH_2- region of Cl30-PCL.

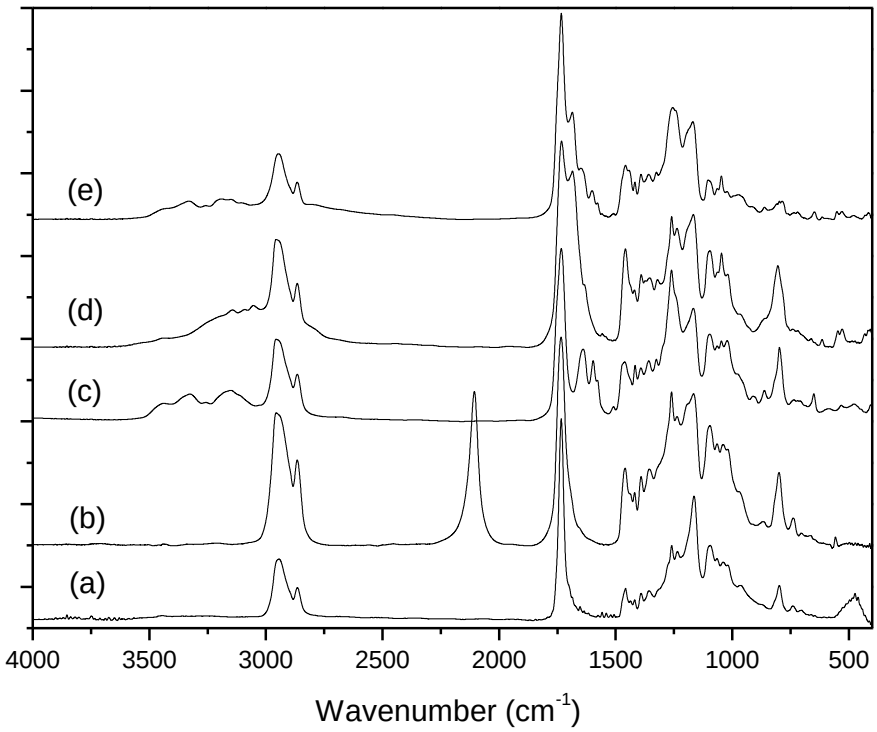

Figure S5. IR spectra of (a) poly(*α*Cl*ε*CL-*co*-εCL) (b) poly(*α*Az*ε*CL-*co*-*ε*CL) (c) poly(*ε*CL-*g*-Uracil) (d) poly(*ε*CL-*g*-Adenine) (e) poly(*ε*CL-*g*-Adenine/Uracil)

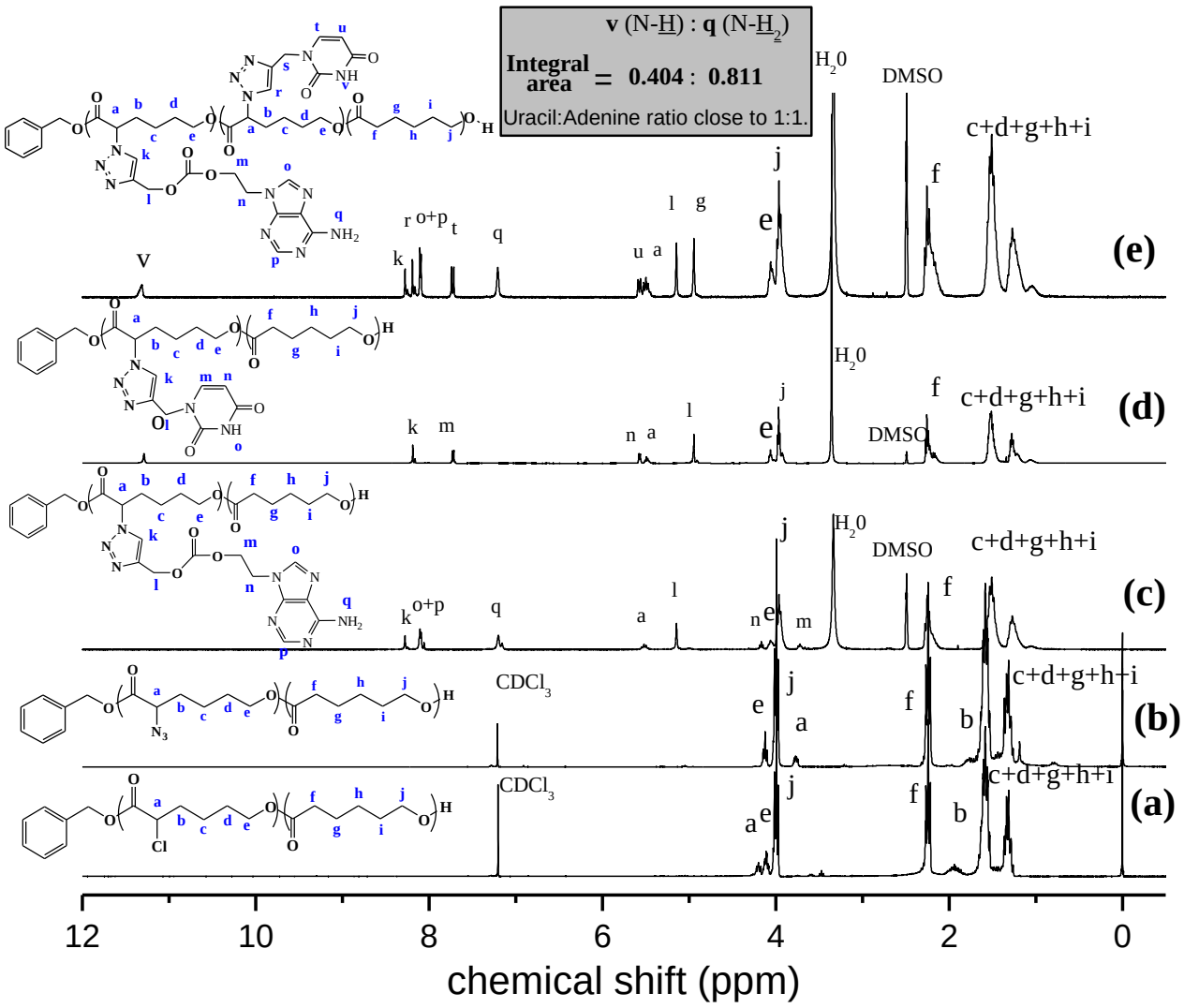

Figure S6. ^{1}H NMR spectra of (a) poly(*α*Cl*ε*CL-*co*-εCL) (b) poly(*α*A*z**ε*CL-*co*-*ε*CL) (c) poly(*ε*CL-*g*-Adenine) (d) poly(*ε*CL-*g*-Uracil) (e) poly(*ε*CL-*g*-Adenine/Uracil)

Table S1. Properties of the poly(*ε*CL-*g*-Adenine), poly(*ε*CL-*g*-Uracil), poly(*ε*CL-*g*-Adenine/Uracil) copolymers.

Entry	Poly(nucleobase-*co*-*ε*CL)			Thermal properties	
	*α*Cl*ε*CL/*ε*CL[a]	M_n GPC[b]	M_w/M_n[b]	T_d,[c] °C	T_g,[d] °C
U30-PCL	32/68	37598	4.9	277	15.2
A40-PCL	45/55	31742	6.6	255	23.3
A30-PCL	30/70	28511	4.5	260	15.8
A20-PCL	18/82	46598	4.2	242	-8.3
A10-PCL	11/89	34491	3.5	257	-9.5
AU30-PCL	32/68	36977	3.6	268	25.4
Cl30-PCL	29/71	17852	1.5	320	-50.8
Az30-PCL	29/71	18663	1.5	317	-59.4

[a] Obtained from integration of the signal at 4.2–4.3 ppm (poly(*α*Cl*ε*CL-*co*-*ε*CL), 1H per repeating unit, –CO–Cl*H*–) and 4.1–4.2 ppm (poly(*α*Cl*ε*CL-*co*-*ε*CL), 2H per repeating unit, –COO–CH_2–).

[b] Obtained from GPC trace (eluent: DMF; 0.6 mL/min; PS-standard calibration): M_n, number-average molecular mass with the highest RI intensity; PDI, molecular mass distribution.

[c] Obtained from TGA thermograms recorded at heating rate of 20 °C/min.

[d] Obtained from second-run DSC thermograms recorded at a heating rate of 20 °C/min

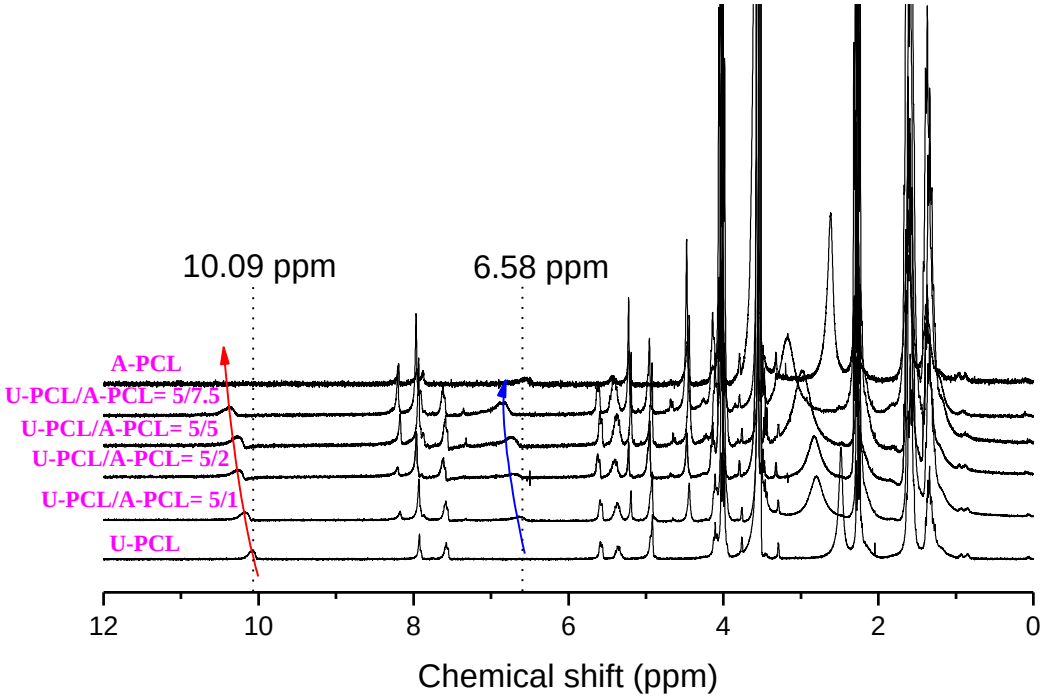

Figure S7. ^{1}H NMR titration of U30-PCL with A10-PCL in 1,4-dioxane-d_8. The chemical shift of NH group of uracil was monitored.

WAXD Analyses. In our previous study,[7] these nucleobase units in the complexes indeed formed physically cross-linked structure via biocomplementary hydrogen bonds. Further investigation on the self-assembly of these complexes was carried out using WXRD measurements (Figure S8). However, careful comparison shows that both (110) and (200) peaks were a significant reduction in intensity and several amorphous halos appearing at 2θ of 19.0° (d = 0.41 nm), 29.3° (d = 0.37 nm), and 40.49° (d = 0.22 nm), indicating that the crystalline PCL became non-crystalline by the attachment of the nucleobases and triazole rings.[8] This observation suggested that the introduction of U-U interaction expands the intermolecular main chain spacing and inhibits the PCL crystallization. These diffractions can be well fitted in the Gaussian function as shown in Figure S8 (g). In addition, WAXD patterns of A30-PCL and AU30-PCL are also shown in Figure S8. These results are similar to those of the U30-PCL.

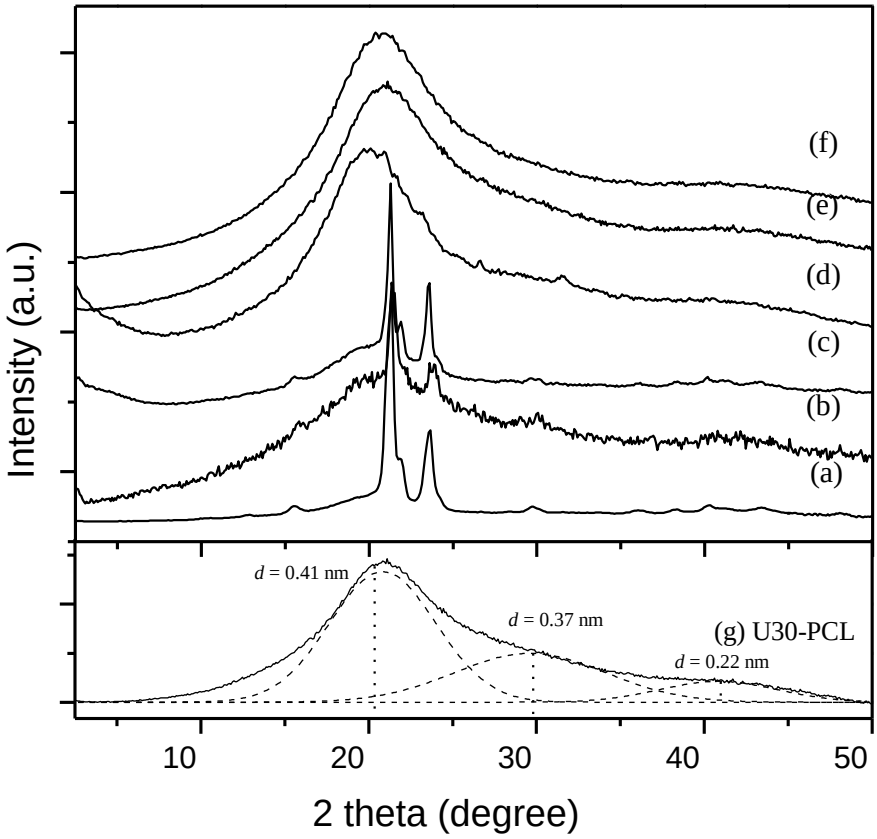

Figure S8. The XRD curves: (a) linear PCL (b) Cl30-PCL (c) Az30-PCL (d) U30-PCL (e) A30-PCL (f) AU30-PCL (g) curve fitting of U30-PCL

FT-IR Spectroscopic Analyses. Infrared spectroscopy is a highly effective means of investigating the specific interactions between polymers at various temperatures (from 25 to 180 °C). Figure S9 (A) illustrates FTIR spectra in the N-H stretching region of the U30-PCL/A30-PCL where the band at 3485 cm^{-1} corresponds to free NH stretching and the peaks at 3320 and 3200 cm^{-1} are attributed to A-U interactions. At 25 °C, the N-H stretching region [Figure S9 (A)] shows a number of broad absorption bands in 3200-3400 cm^{-1} region. Vibration at 3320 and 3200 cm^{-1} correspond to medium strength hydrogen-bonded NHs,[9] implying that U30-PCL/A30-PCL indeed forms hydrogen-bonded interaction in the solid state. Figure S9 (B) displays FTIR spectra (1500-1800 cm^{-1}) for A30-PCL blended with the U30-PCL copolymer. Three peaks observed at ca. 1678 cm^{-1} (C=O group of U interacting with A) and 1642 cm^{-1} (bonded NH_2 scissor plus ring stretching), and 1600 (ring stretching plus bonded NH_2 scissor). Upon heating from 25 to 180 °C, signals at 3320 and 3200 cm^{-1} (corresponding to N-H stretching) shifted toward higher wave number and the free N-H stretching vibration at 3485 cm^{-1} appears gradually, indicating the change in the nature of the hydrogen bonding at high temperature. However, the purine group at 1642 and 1600 cm^{-1} shifts to lower wavenumber, consistent with the heat destruction of a hydrogen bond of a pyrimidine group. At temperatures higher than T_g of the blend, a new peak appears at ca. 1586 cm^{-1}, corresponding to a free NH_2 scissor vibration plus ring stretching vibration of the pyrimidine group, indicating the dissociation of the multiple hydrogen-bonding interactions between the adenine and uracil groups. In addition, Figure S9 shows the N-H intensity of A30-PCL/U30-PCL blend plotted against the temperature to clearly show the destruction for hydrogen bonding.

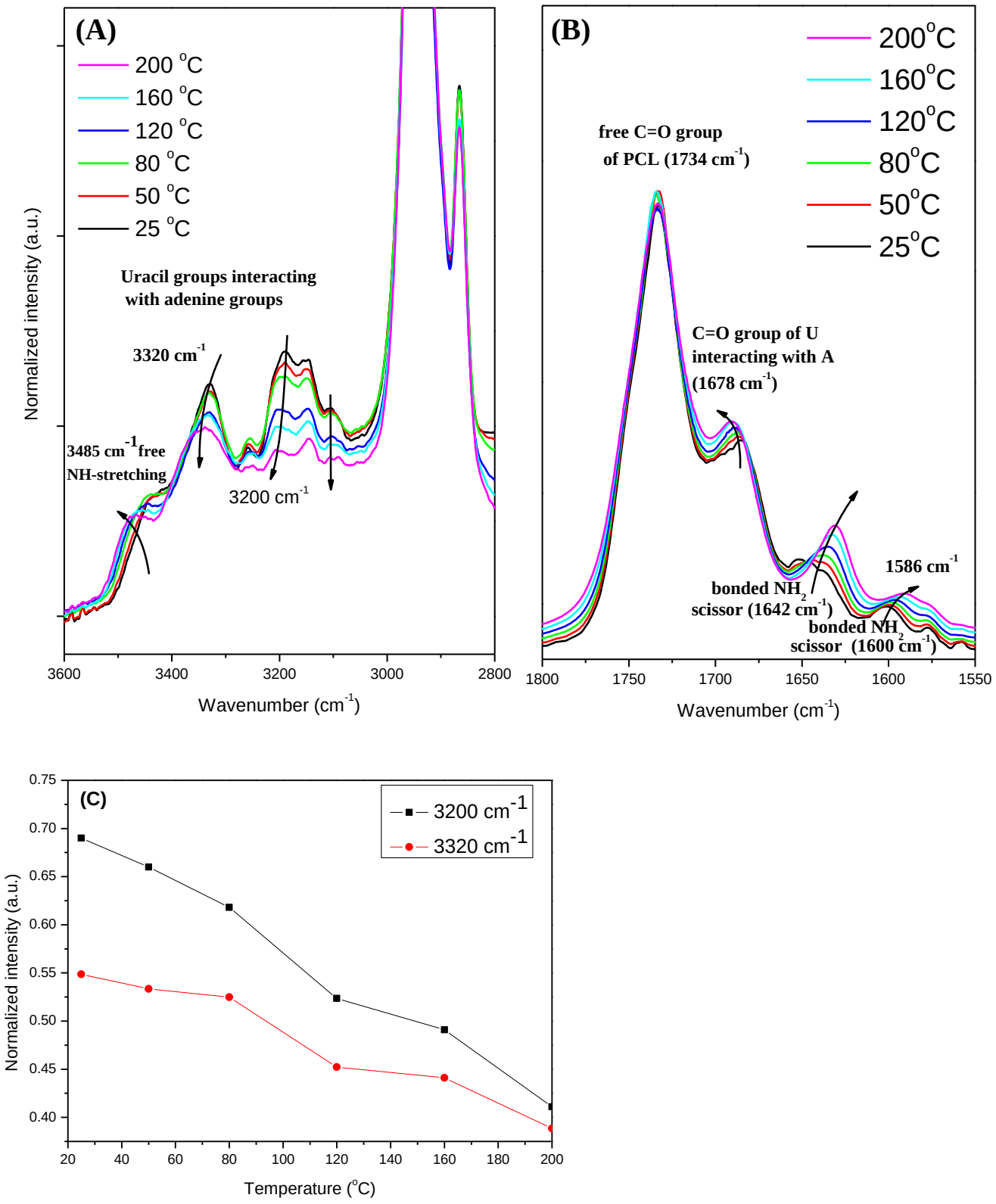

Figure S9. (A,B) FTIR spectra of U30-PCL/A30-PCL blend, recorded at various temperatures. (C) Intensity vs temperature for A30-PCL/U30-PCL.

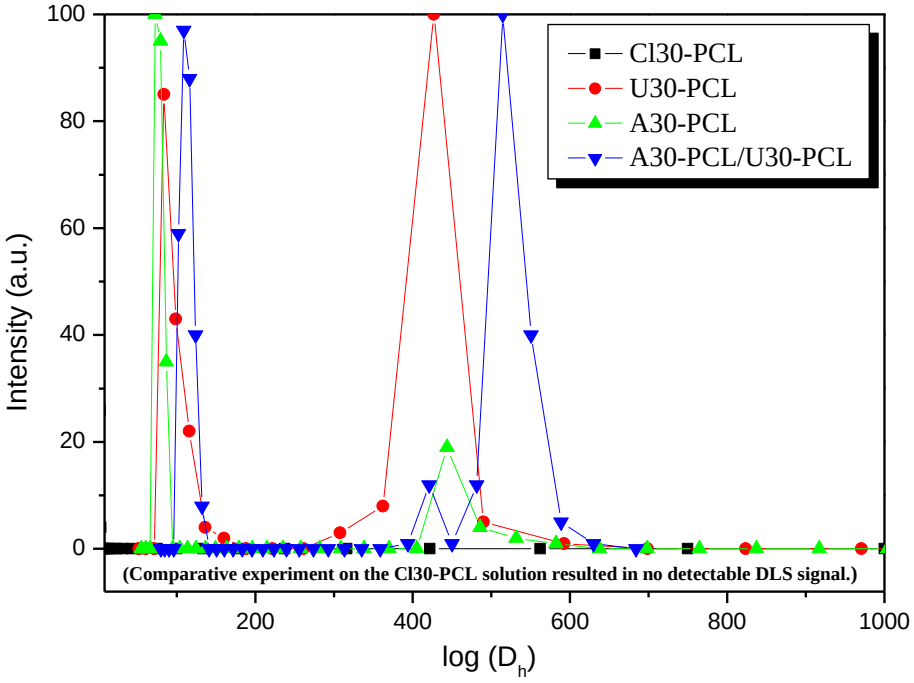

Figure S10. DLS analyses of Cl30-PCL, U30-PCL, A30-PCL, AU30-PCL, and A30-PCL/U30-PCL (Concentration of each sample: 50 mg/L).

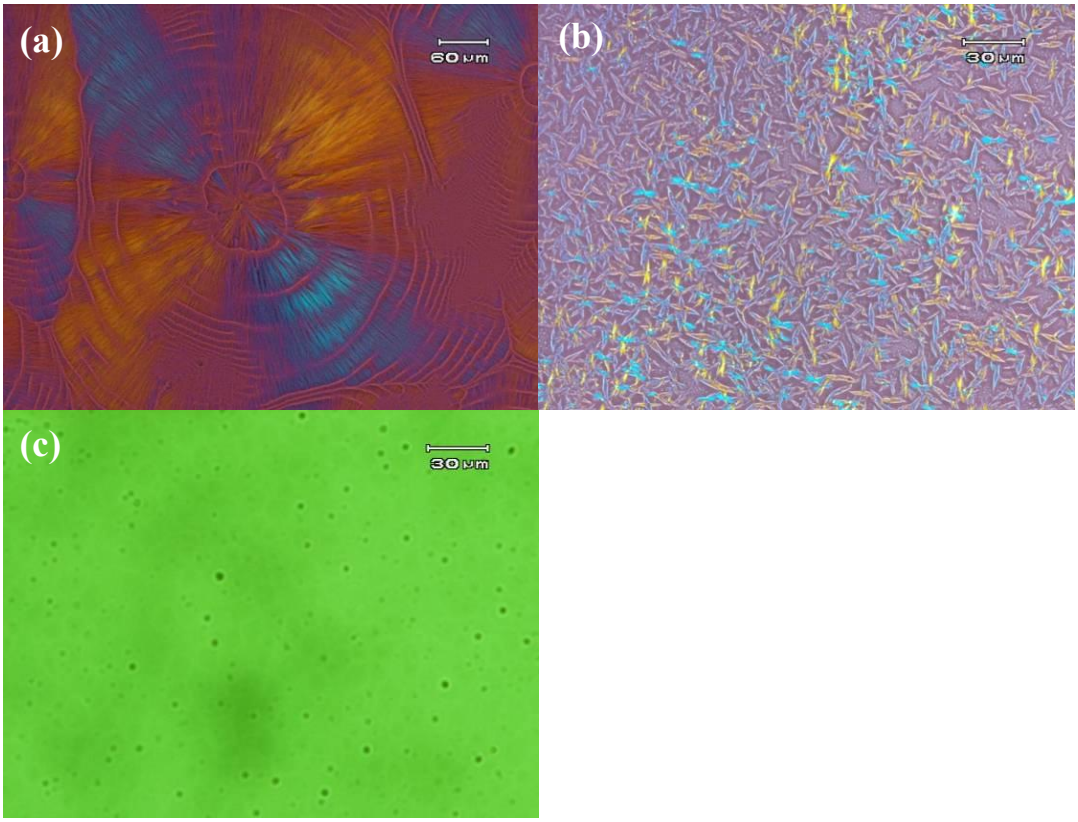

Figure S11. Polarized optical micrographs of (a) linear PCL (b) Az30-PCL (c) A30-PCL.

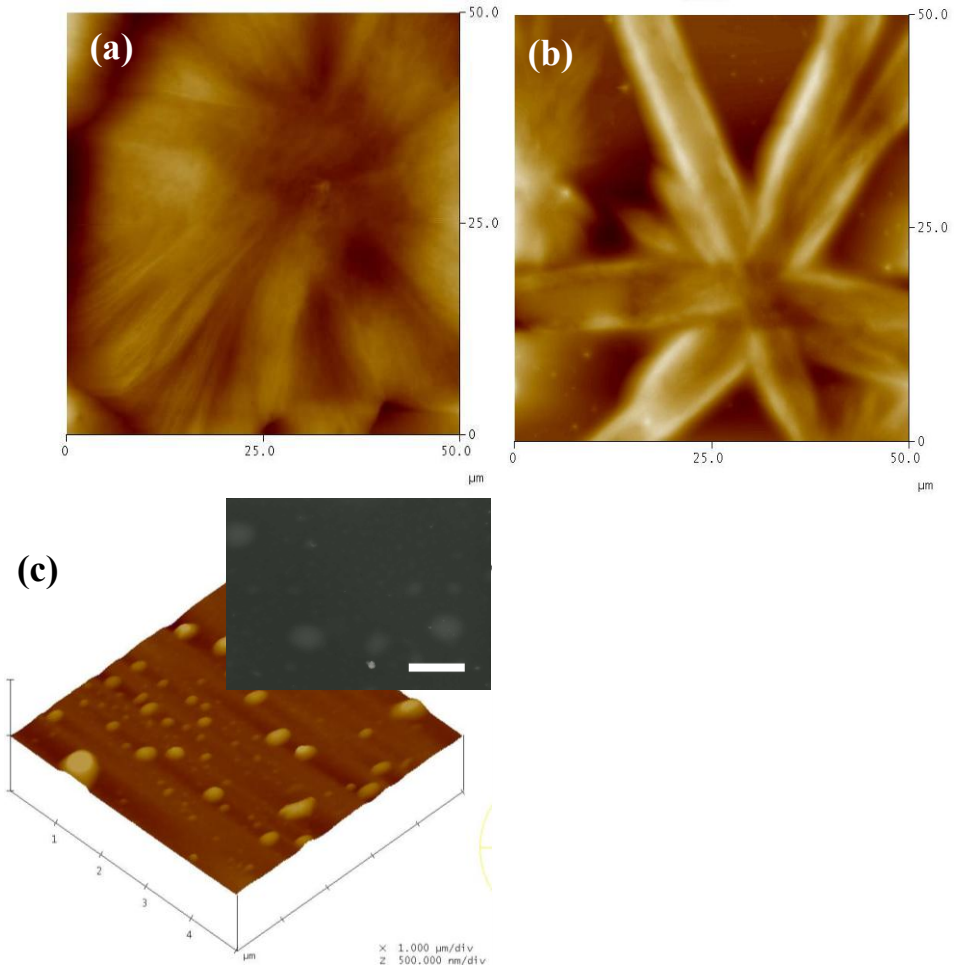

Figure S12. AFM height images of (a) linear PCL (b) Az30-PCL (c) A30-PCL. Shown in the insets are TEM images. The scale bars in TEM images: 1 µm.

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