Electronic Supplementary Information (ESI)

Non-ionic water-soluble "clickable" α-helical polypeptides: synthesis, characterization and side chain modification

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Experimental

Materials. All the chemicals and Dowex® Marathon® C resin were all purchased from Sigma-Aldrich and used as received unless specified. Z-Lys-OH was purchased from AAPPTec, LLC. Benzylamine was first stirred with calcium hydride overnight, and then distilled under vacuum. Anhydrous solvents were purified by passing through alumina columns under argon. NCA monomers precursors γ-propargyl L-glutamate hydrochloride and N-ε-2-[2-(2-Methoxyethoxy)ethoxy]acetyl-N-α-Z-L-Lysine were synthesized according to reported procedures.[1, 2] Azido terminated GRGDS was synthesized according to a reported procedure.[3]

Instrumentation. ^{1}H NMR spectra were recorded on a Bruker AV-400 or AV-500 spectrometer. Chemical shifts in parts per million (ppm) were referenced relative to proton impurities or ^{13}C isotopte of deuterated solvents (e.g., $CDCl_3$). SEC-DRI analyses were performed with an Agilent 1200 system equipped with three Phenomenex 5 μm, 300 × 7.8 mm columns [100 Å, 1000 Å and Linear(2)], Wyatt DAWN EOS multi-angle light scattering (MALS) detector (GaAs 30 mW laser at λ=690 nm) and Wyatt Optilab rEX differential refractive index (DRI) detector with a 690 nm light source. DMF containing 0.1 M LiBr was used as the eluent at a flow rate of 0.5 mL·min^{-1}. The temperature of the column and detector was 25 °C. Polymer molecular weight (M_n) and molecular weight distribution (PDI) were obtained by conventional SEC analysis with a calibration curve built using polystyrene standards. Circular dichroism (CD) data were collected on a Jasco J810 CD spectrometer (Japan Spectroscopic Corporation) with a path length of 0.1 cm and a band width of 1 nm at 20 °C. Three scans were collected and averaged between 190 nm and 250 nm at a scanning rate of 50 nm·min^{-1} with a resolution of 1 nm. The content of various secondary structures were calculated by DICHROWEB using Contin-LL. FTIR spectra were collected with a Bruker Alpha FT-IR spectrometer. Dynamic light scattering (DLS) analysis was conducted on a Malvern Zetasizer Nano-ZS instrument using zetasizer software 6.12.

Cell adhesion assay. Cell adhesion on immobilized human fibrinogen and GRGDS conjugated polymers was assessed by the measurement of cellular lactate dehydrogenase (LDH) activity. Briefly, Chinese hamster ovary cells (CHO cells) stably expressing integrin $\alpha_{IIb}\beta_3$ were maintained in MEM-α medium (Life Technologies, Grand Island, NY) supplemented with 10% FBS, 1% None-Essential Amino Acids, 1% L-Glutamine, 1% Sodium Pyruvate and 1% Penicillin-Streptomycin (All from Life Technologies, Grand Island, NY). Before the assay, cells were first detached by trypsin-EDTA and suspended in HBS supplemented with 5.5 mM glucose and 1% bovine serum albumin and 1 mM Ca^{2+} were seeded on flat bottom 96-well plates (~5000 cells/well) pre-coated with GRGDS conjugated polymers or fibrinogen at the concentrations ranged from 1.5 μg/mL to 50 μg/mL and blocked with 1% bovine serum albumin. After incubation at 37 °C for 30 min, wells were washed three times with ice-cold PBS. Remaining

adherent cells were lysed with 1% Triton X-100, and LDH activity was assayed using the Cytotoxicity LDH Detection Kit (CloneTech Laboratories Inc., CA) according to the manufacturer's instructions. The 492 nm absorbance of formazan was measured by a BIO-RAD microplate reader (Model 680, Life Science Research, CA). Cell adhesion was expressed as a percentage of bound cells relative to total input cells.

Synthesis of γ -propargyl L-glutamatic acid *N*-carboxyanhydride (PLG NCA) (M1). PLG NCA was synthesized by adapting a published procedure.[1] Briefly, γ-propargyl L-glutamatic acid hydrochloride (3.00 g, 13.5 mmol) was suspended in anhydrous THF (100 mL) at 50 °C under nitrogen, triphosgene (1.64 g, 5.52 mmol) was added to the above suspension under nitrogen flow. After the heterogeneous solution turned clear within 3 h, THF was removed under vacuum to afford a clear oil product. The oily product was purified by flash chromatography first with anhydrous hexanes and then gradient anhydrous THF/hexanes (9:1 ~ 1:1 v/v) mixtures (R_f = 0.23 in 1 : 1 THF/hexane). Fractions containing the desired product were combined and concentrated to afford a clear oil which solidified upon storage at - 20 °C inside glovebox (1.40 g, 49% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 6.69 (s, 1H), 4.73 (d, 2H), 4.46 (t, 1H), 2.63 (t, 2H), 2.54 (t, 1H), 2.31-2.18 (m, 2H). See Figure S14.

Synthesis of N-ε-2-[2-(2-Methoxyethoxy)ethoxy]acetyl-L-lysine *N*-carboxyanhydride (EG_2-LYS NCA) (M2). EG_2-LYS NCA was synthesized by adapting a published procedure.[2] Briefly, N-*ε*-2-[2-(2-Methoxyethoxy)ethoxy]acetyl-N-*α*-Z-L-Lysine (6.02 g, 13.7 mmol) was dissolved in anhydrous CH_2Cl_2 (200 mL) in an oven dried round bottom flask equipped with a stir bar under nitrogen flow. 1,1-Dichloromethylmethyl ether (6.80 g, 59.2 mmol) was added to the above solution. The reaction mixture was heated to at 45 °C and allowed to reflux under nitrogen for 12 h during which time a 90% conversion was reached. The reaction mixture was then concentrated under vacuum using a Schlenk line to afford a clear oil. The crude product was first purified by recrystallization in anhydrous THF/hexanes in glovebox at - 20 °C to afford a white solid. The white solid was further purified by dry flash chromatography under nitrogen flow. Column was eluted with gradient anhydrous THF/hexanes (2:1 ~ 1:0 v/v). Fractions containing the desired product were combined and the volatiles were removed to yield a white solid as the final product (1.60 g, 35% yield) (R_f = 0.34 in THF). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.68 (s, 1H), 7.33 (s, 1H), 4.30 (m, 1H), 4.00 (m, 2H), 3.70-3.59 (m, 8H), 3.39-3.31 (m, 5H), 2.02-1.81 (m, 2H), 1.64-1.49 (m, 4H). See Figure S15.

Synthesis of benzyl azide. Benzyl azide was synthesized by adapting a published procedure.[4] Benzyl bromide (5.45 g, 31.9 mmol) was dissolved in DMF (460 mL) in a round bottom flask under nitrogen. Sodium azide (5.28 g, 81.2 mmol) was then added to the above solution. The reaction was stirred at room temperature for 1 h during which a full conversion was reached. Diethyl ether (300 mL) was added to the reaction mixture. The organic phase was washed with DI water 6 times (6×100 mL), separated and dried over anhydrous $MgSO_4$. Filtration and removal of volatiles under vacuum yielded a clear oil as the product (2.99 g, 70% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.35 (m, 5H), 4.30 (s, 2H).

Synthesis of octyl azide. Octyl azide was synthesized in a similar manner as benzyl azide by adapting a published procedure.[4] 1-Bromooctane (6.08 g, 31.5 mmol) was dissolved in DMF (240 mL) in a round bottom flask under nitrogen. Sodium azide (5.13 g, 78.9 mmol) was then added to above solution. The reaction was at room temperature overnight. Diethyl ether (300 mL) was added to the reaction mixture. The organic mixture was washed with DI water (1200 mL) 6 times, separated and dried over anhydrous $MgSO_4$. Filtration and removal of volatiles under vacuum afforded a clear oil as the product (4.37 g, 89% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 3.25 (m, 2H), 1.60 (m, 2H), 1.28 (m, 10H), 0.88 (t, 3H).

Representative procedure for synthesis of polypeptide P(PLG_{17}-r-PLL_{69}). PLG NCA (150.7 mg, 0.714mmol) and EG_2-LYS NCA (0.9548 g, 2.88 mmol) were dissolved in anhydrous DMF (8.3 mL) inside a glovebox. A measured volume of DMF stock solution of benzylamine (668 μL, 0.0359 mmol, 53.8 mM) was added to the above solution. The reaction was stirred at 50 °C inside a glovebox for 12 h. Diethyl ether was added to the above solution at room temperature to yield an oily precipitate. The crude oily product was then re-dissolved in DI water (~10 mL) and transferred into a centrifugal dialysis tube (10 kDa MWCO). The centrifugal tube was spun at 3000 rpm till all of the water went through the filter membrane. The residue polymer sample was then re-dissolved in DI water for repeated dialysis (3 times). Aqueous solution obtained after dialysis was lyophilized to give a clear sticky solid (0.68 g, 72% yield). ^{1}H NMR and ^{13}C NMR spectra are shown in Figure S1 and S2 respectively.

Representative procedure for the grafting of P(PLG_m-r-PLL_n) with hydrophobic moieties by CuAAC. P(PLG_{17}-r-PLL_{69}) (91.2 mg, 0.00402 mmol) and octyl azide (13.5 mg, 0.0871 mmol) were dissolved in anhydrous

DMF (1.34 mL). PMDETA (14.2 mg, 0.0821 mmol) were added to the above solution together with a half inch length of freshly filed copper wire. The reaction was stirred at 50 °C under nitrogen for 24 h. The copper wire was removed by filtration. The remaining clear light blue solution was diluted with THF (~ 10 mL), added with Dowex® Marathon® C resin (0.7 g) and stirred for 24 h to remove residual Cu (II) ion. Removal of the resin by filtration yielded a clear solution which was further concentrated under a flow of nitrogen. Addition of diethyl ether to the concentrate produced a white precipitate, which was collected by centrifugation and dried under vacuum (35.8 mg, 35% yield). ^{1}H NMR and ^{13}C NMR spectra are shown in Figure 2 and Figure S16.

Representative procedure for the grafting of P(PLG$_m$-r-PLL$_n$) with N$_3$-GRGDS by CuAAC. P(PLG$_{14}$-r-PLL$_{72}$) (65.1 mg, 0.00282 mmol) and N$_3$-GRGDS (25.7 mg, 0.0409 mmol) were dissolved in anhydrous DMF (1 mL). PMDETA (3.3 mg, 0.019 mmol) were added to the above solution together with a half inch length of freshly filed copper wire. The reaction was stirred at 50 °C under nitrogen for 24 h. The copper wire was removed by filtration. DMF was removed by vacuum distillation. The residue solid was then re-dissolved in an edetate disodium (EDTA) (0.4g, 1mmol)/DI water (30 mL in total) solution, transferred into a centrifugal dialysis tube (10 kDa MWCO). The centrifugal tube was spun at 3000 rpm till all of the aqueous solution went through the filter membrane. The residue polymer sample was then re-dissolved in 3 mL DI water to repeat the centrifugal dialysis 3 times. The aqueous solution obtained after dialysis was lyophilized to give a white solid (28.2 mg, 31% yield). ^{1}H NMR spectrum is shown in Figure S14.

Table S1: Benzylamine-initiated ROP of PLG NCA (M_1) and EG$_2$-LYS NCA (M_2) in different feed ratios to yield the P(PLG$_m$-r-PLL$_n$) polypeptides.

Entry #	Polymer composition [a]	$[M_1]_0/[M_2]_0/[I]_0$	M_n (theo.) [b] (kg·mol^{-1})	M_n (NMR) [a] (kg·mol^{-1})	M_n (SEC) [c] (kg·mol^{-1})	PDI [c]	conv. (%)
1	P(PLG$_{14}$-r-PLL$_{72}$)	19/81/1	26.5	23.1	30.7	1.23	100[d], 100[e]
2	P(PLG$_{17}$-r-PLL$_{69}$)	20/80/1	26.4	22.7	35.4	1.39	100[d], 100[e]
3	P(PLG$_{45}$-r-PLL$_{47}$)	49/51/1	22.9	21.1	34.4	1.32	100[d], 100[e]
4	P(PLG$_{70}$-r-PLL$_{18}$)	79/21/1	18.0	16.9	28.4	1.24	98[d], 84[e]

[a.] determined by ^{1}H NMR spectroscopy; [b.] theoretical molecular weight based on single-site initiation; [c.] determined by SEC-DRI in 0.1M LiBr/DMF using polystyrene standards; [d.] conversion for [M_1] determined by ^{1}H NMR spectroscopy; [e.] conversion for [M_2] determined by ^{1}H NMR spectroscopy.

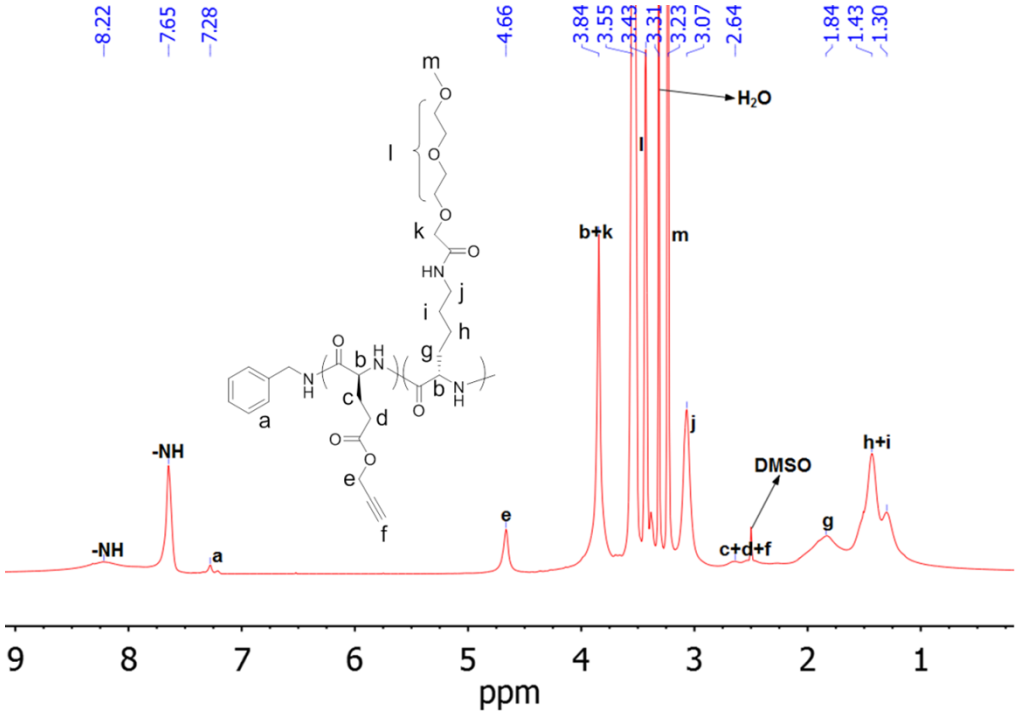

Figure S1. ^{1}H NMR spectrum of the P(PLG$_{17}$-r-PLL$_{69}$) copolymer in DMSO-d$_6$.

(Note: the copolymer composition is calculated based on the ratio of methylene protons of the PPLG segments **e** and the methylene protons of PPLL segment **j** relative to the aromatic protons of the benzyl end group **a**).

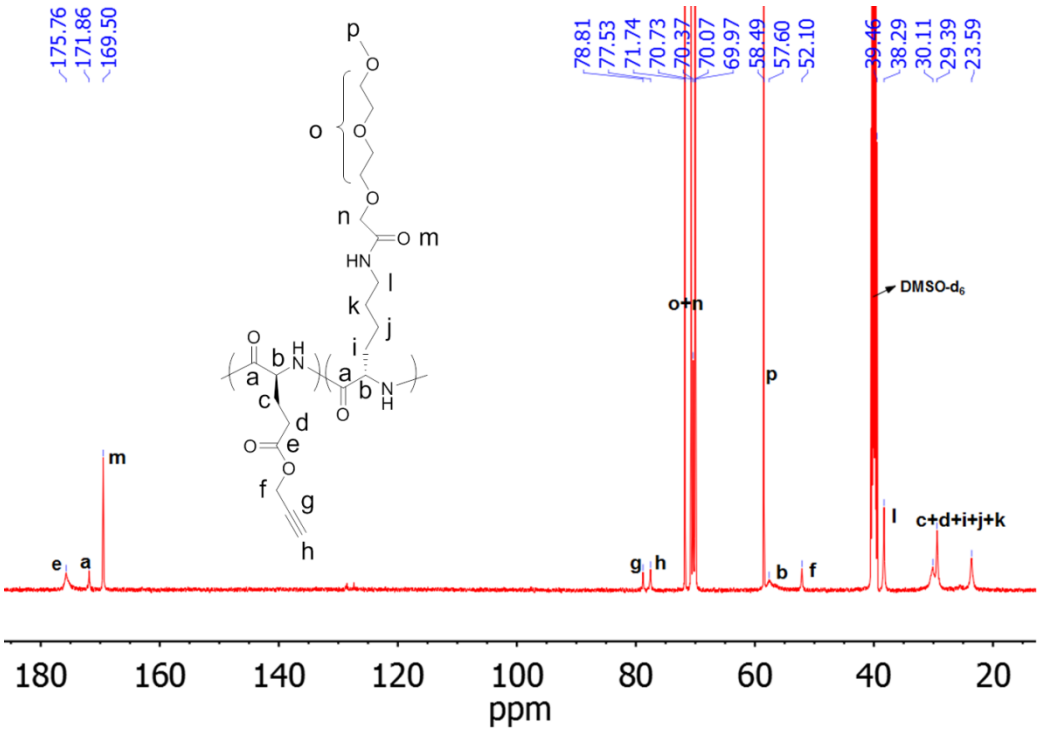

Figure S2. ^{13}C NMR spectrum of the P(PLG$_{17}$-r-PLL$_{69}$) copolymer in DMSO-d$_6$.

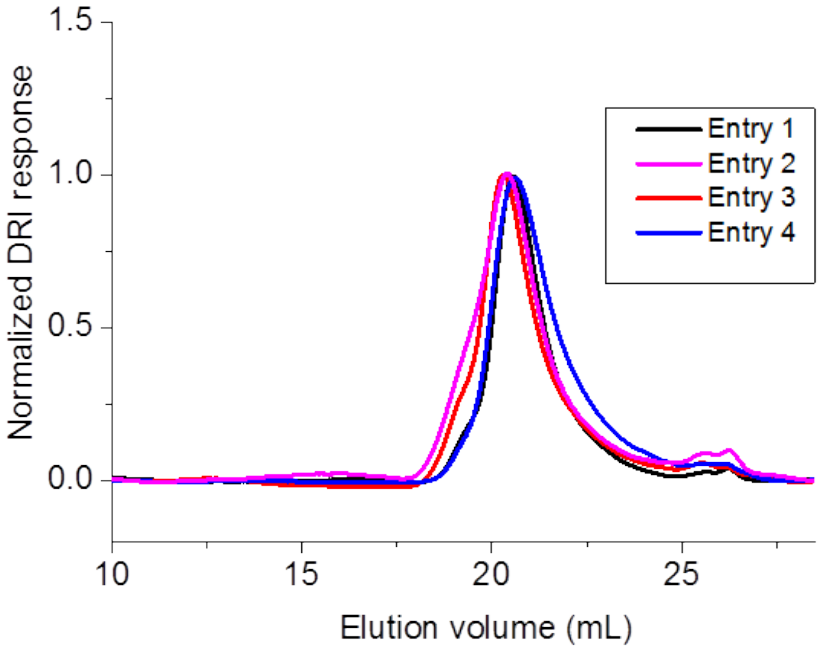

Figure S3. SEC-DRI chromatograms of P(PLG_m-r-PLL_n) polymers (Entry 1-4, Table S1) in 0.1 M LiBr/DMF. (Note: the polymerization reaction mixtures were analyzed directly by SEC-DRI after high conversions were reached).

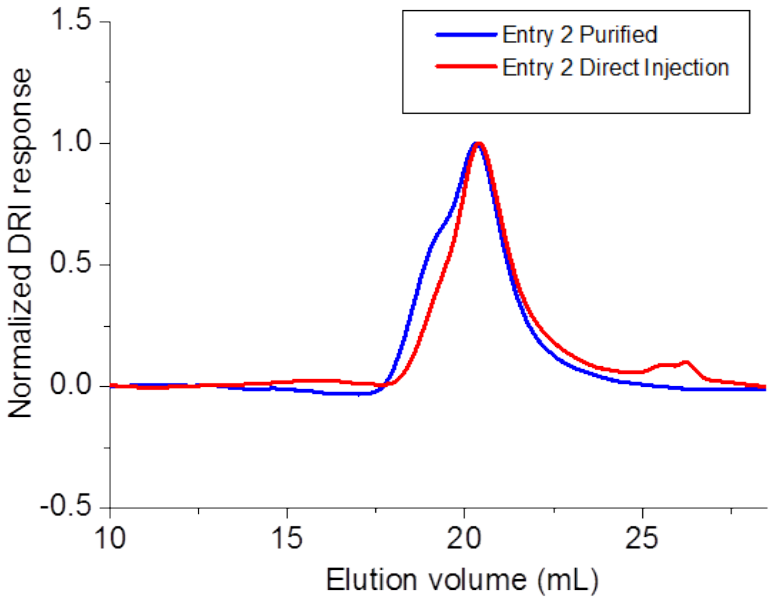

Figure S4. SEC-DRI chromatograms of the P(PLG_{17}-r-PLL_{69}) copolymers (Entry 2, Table S1) in 0.1 M LiBr/DMF.

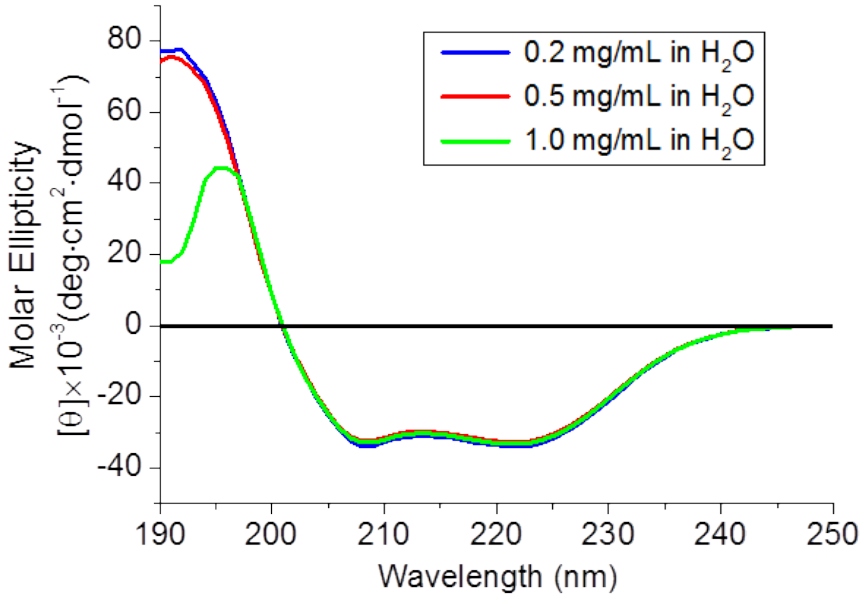

Figure S5. CD spectra of the P(PLG_{17}-r-PPL_{69}) copolymer at different concentrations in H_2O.

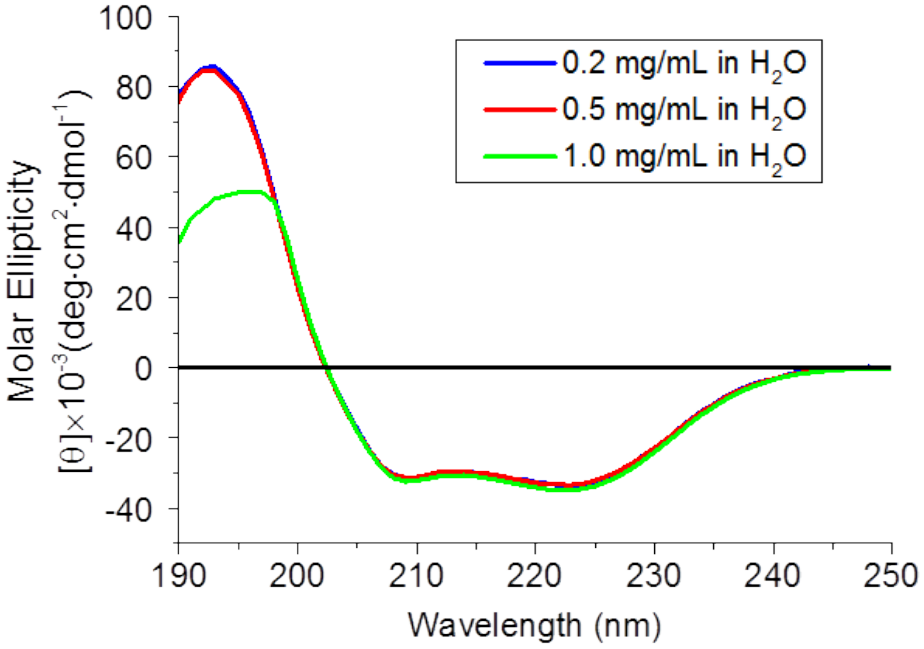

Figure S6. CD spectra of the P(PLG_{45}-r-PPL_{47}) copolymer at different concentrations in H_2O.

Table S2. Secondary conformation analysis of polypeptides using Dichroweb.[5, 6]

Polymer composition	α-helix	β-strand	turn
P(PLG$_{14}$-r-PPL$_{72}$)	89.0%	3.7%	7.3%
P(PLG$_{17}$-r-PPL$_{69}$)	84.5%	4.1%	11.4%
P(PLG$_{45}$-r-PPL$_{47}$)	94.2%	1%	4.7%
P((PLG$_{17}$-g-Oct)-r-PPL$_{69}$)	92.6%	1.8%	5.6%
P((PLG$_{17}$-g-Bn)-r-PPL$_{69}$)	83.7%	3.1%	13.1%
P((PLG$_{14}$-g-GRGDS$_8$)-r-PPL$_{72}$)	99.1%	0.8%	0%

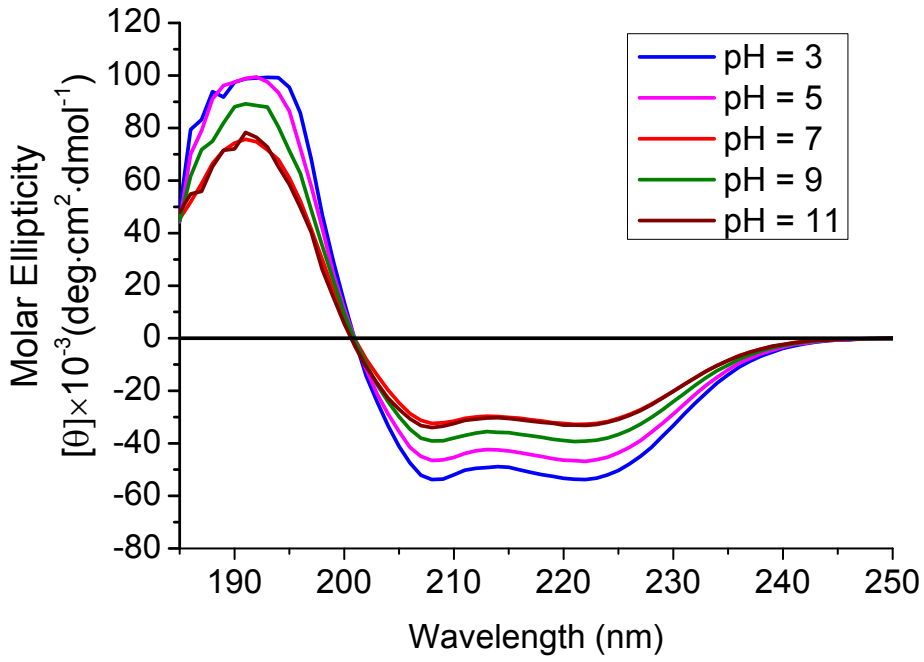

Figure S7. CD spectra of the P(PLG$_{17}$-r-PPL$_{69}$) copolymer at different pHs in H_2O (0.5 mg/mL). (Note: Notable hydrolysis of the ester linkages on the PPLG side chains were shown to occur to varying extent under strong acidic (pH = 3) or basic conditions (pH =11) during the time frame for the solution preparation for CD measurements. No notable hydrolysis were detected for solution samples at pH 5, 7 and 9 in the same time frame, as evidenced by ^{1}H NMR analysis. In addition to ester hydrolysis, there may be other factors that attribute to the differing CD spectra of P(PLG$_{17}$-r-PPL$_{69}$) at varying pH, which remain to be investigated).

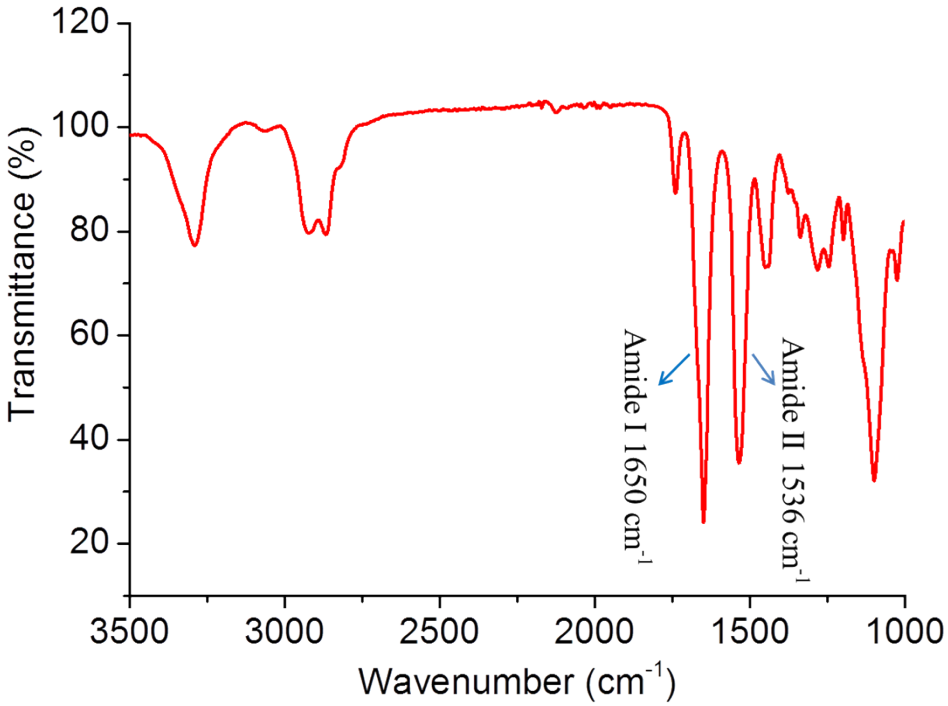

Figure S8. FT-IR spectrum of P(PLG_{17}-r-PPL_{69})

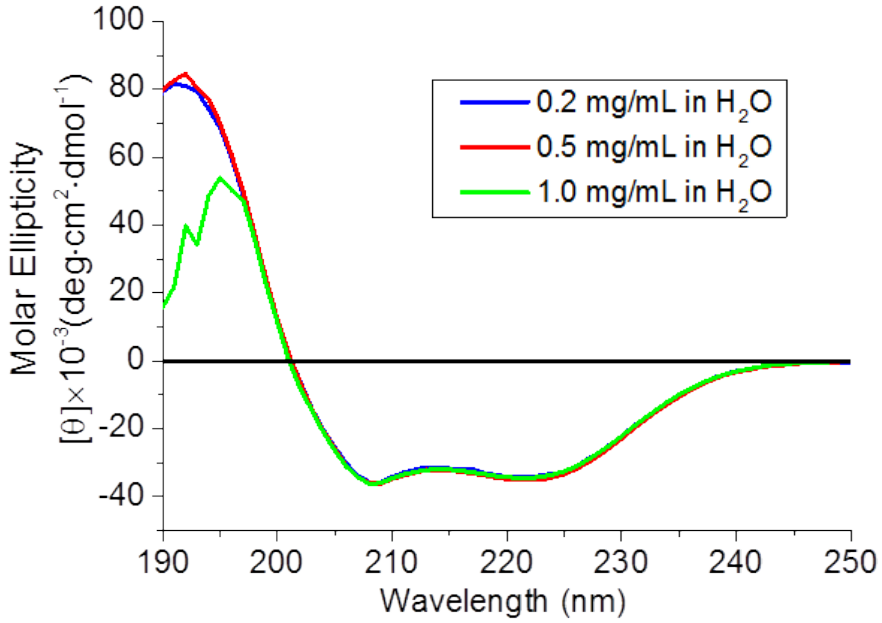

Figure S9. CD spectra of the P((PLG_{17}-g-Oct)-r-PPL_{69}) copolymer at different concentrations in H_2O.

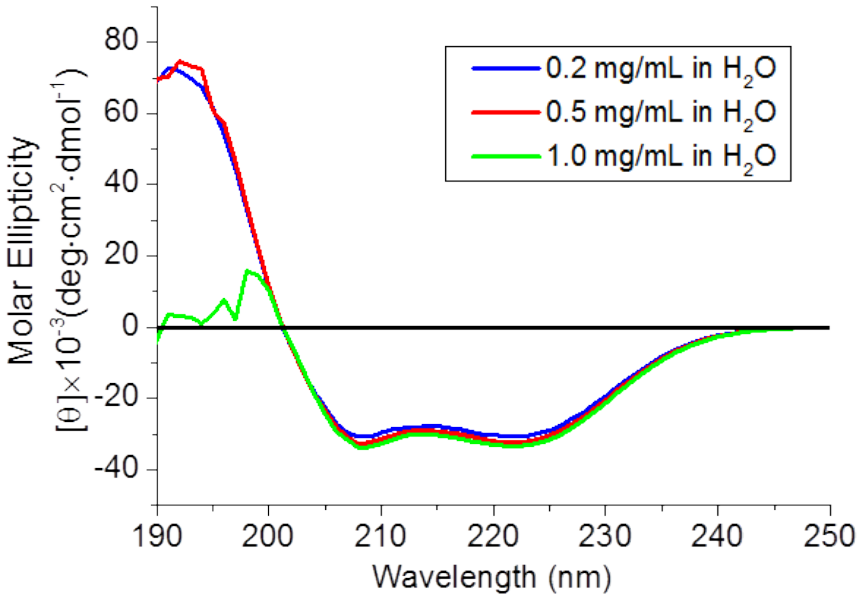

Figure S10. CD spectra of the P($(PLG_{17}$-g-Bn)-r-PPL_{69}) copolymer at different concentrations in H_2O.

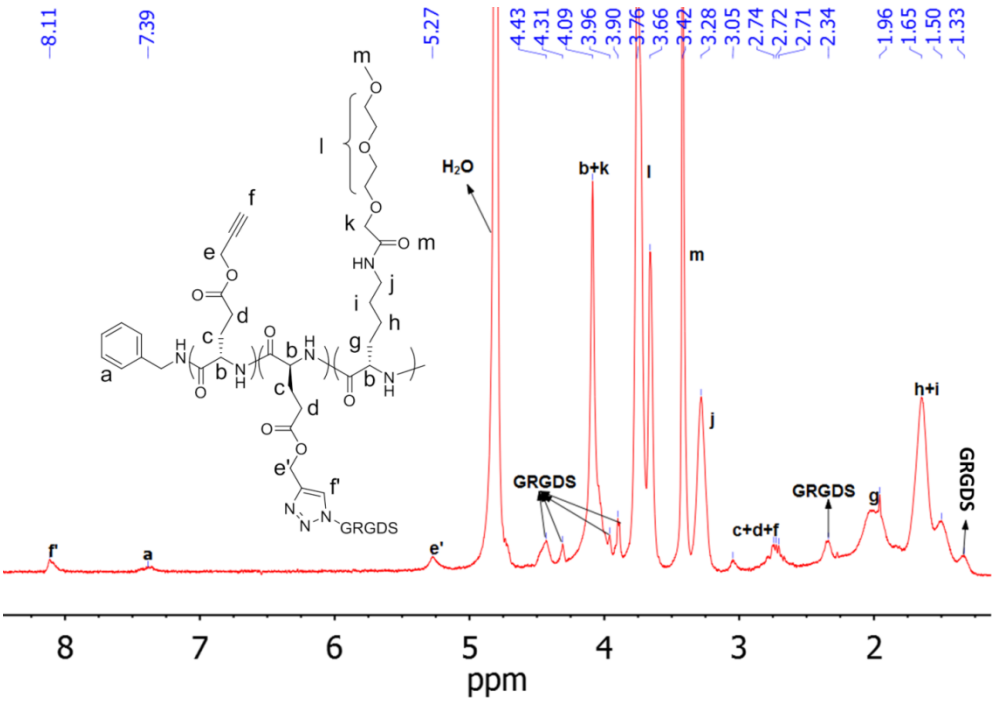

Figure S11. ^{1}H NMR spectrum of P($(PLG_{14}$-g-$GRGDS_8$)-r-PPL_{72}) in D_2O.

(Note: the GRGDS composition in the conjugate is calculated based on the ratio of the triazole proton **f'** relative methylene protons of PPLL segment **j**)

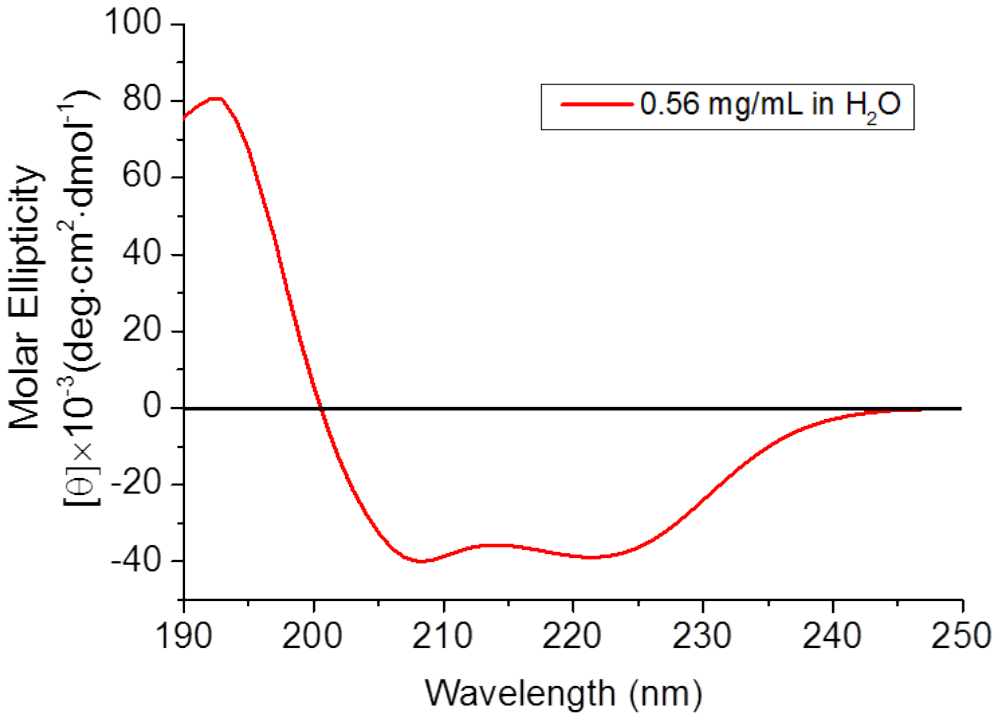

Figure S12. CD spectrum of the P((PLG$_{14}$-g-GRGDS$_8$)-r-PPL$_{72}$) copolymer in H_2O.

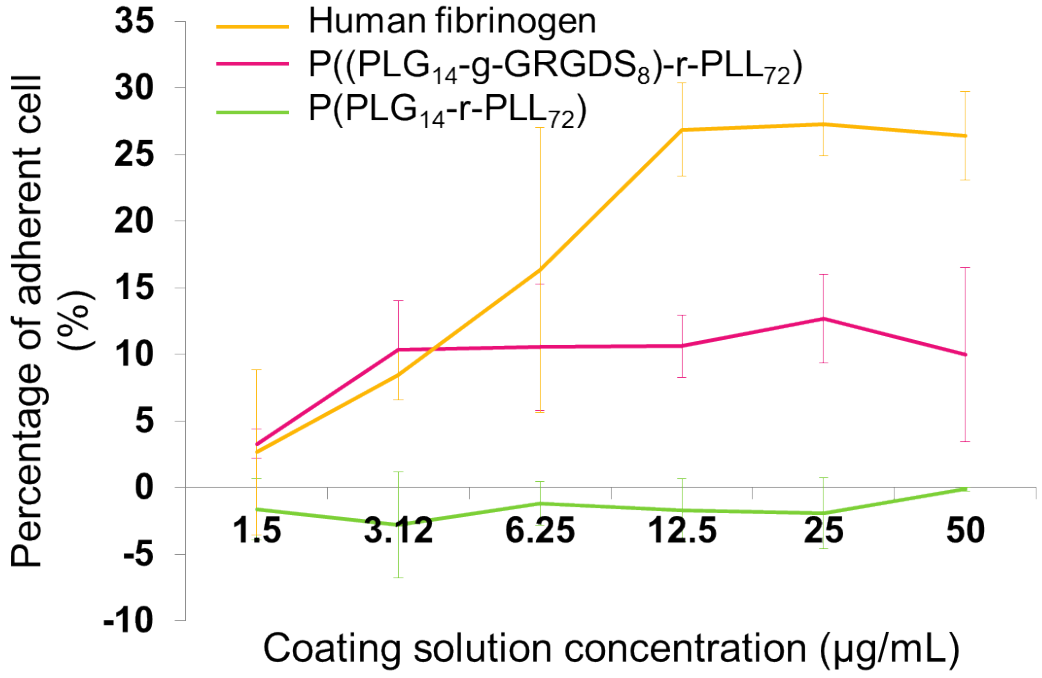

Figure S13. Number percentage of adhered cells versus the concentration of P((PLG$_{14}$-g-GRGDS$_8$)-r-PPL$_{72}$), fibrinogen and P(PLG$_{14}$-r-PPL$_{72}$) used in the coating of the plates.

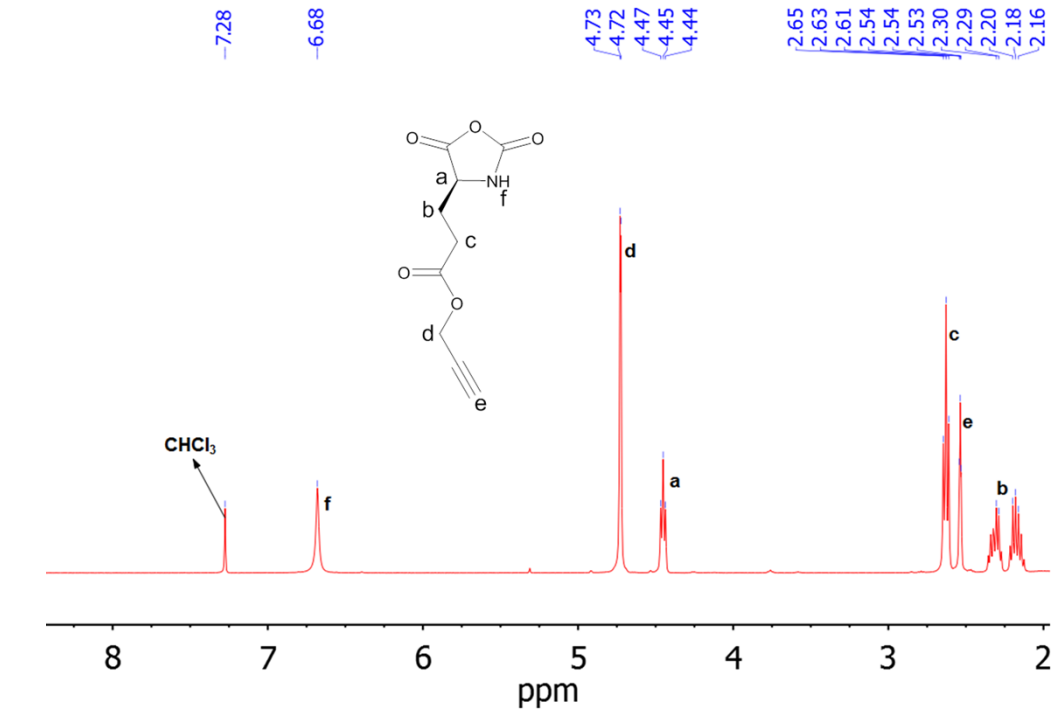

Figure S14. ^{1}H NMR spectrum of PLG NCA in $CDCl_3$.

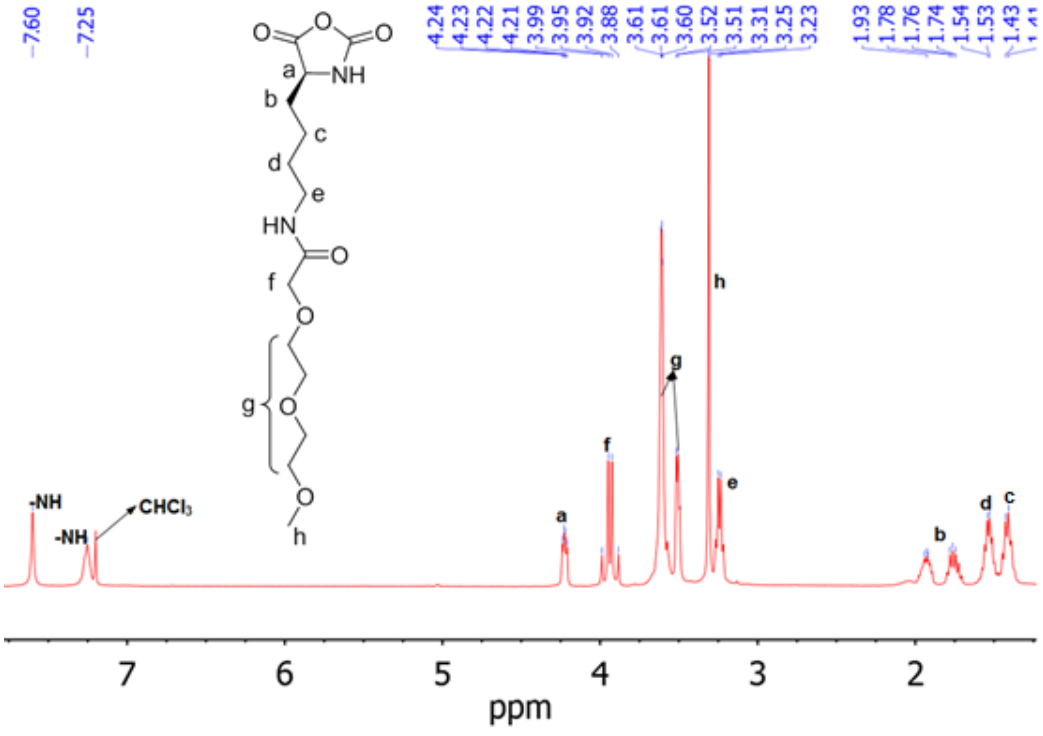

Figure S15. ^{1}H NMR spectrum of EG_2-LYS NCA in $CDCl_3$.

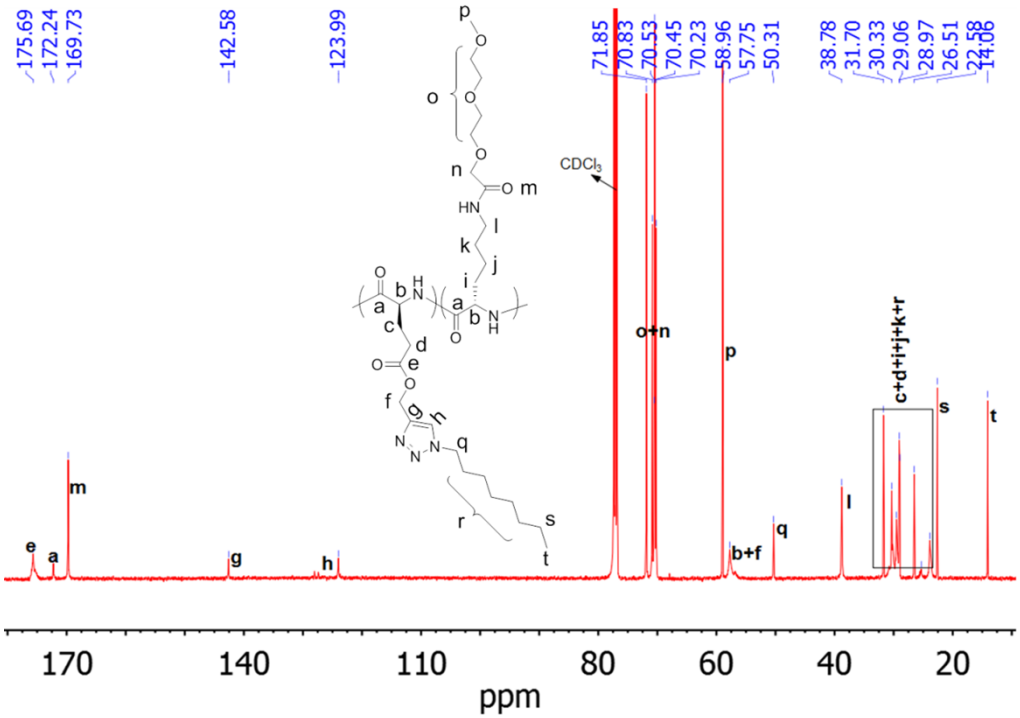

Figure S16. ^{13}C NMR spectrum of the P((PLG$_m$-g-Oct)-r-PPL$_n$) copolymer in $CDCl_3$.

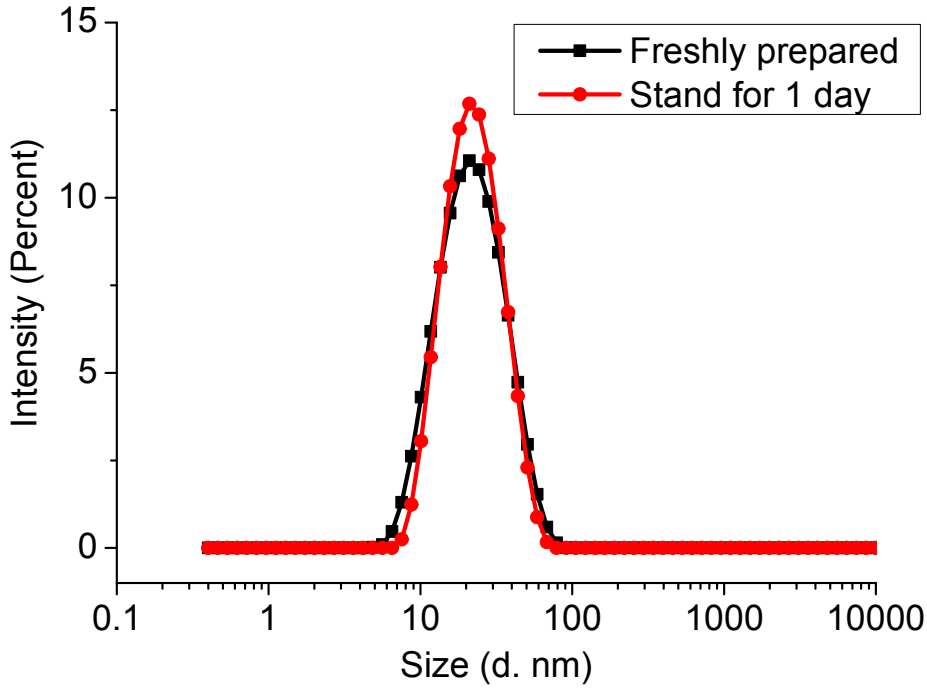

Figure S17. Size distribution of P((PLG$_{17}$-g-Oct)-r-PPL$_{69}$) in Milli-Q water (0.5 mg/mL) measured by DLS. [Note: the hydrodynamic diameter of a freshly prepared sample is 18.8 nm (PDI = 0.205). After the sample was left to stand for 1 d, the hydrodynamic diameter is 19.60 nm (PDI = 0.173). This indicates the particle size is fairly stable].

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