Supporting Information

Topology effect on protein-polymer block copolymer self-assembly

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Materials

$K_3PO_4 \cdot H_2O$ (ACROS Organics), carbone disulfide (Sigma-aldrich), 2-bromoisobutyric acid (Sigma-aldrich), exo-3,6-epoxy-1,2,3,6-tetrahydrophthalic anhydride (Sigma-aldrich), 3-amino-1-puropanol (Sigma-aldrich), 3-amino-1,2-propanediol (Sigma-aldrich), 1-(3-dimethyoaminopropyl)-3-ethylcabodiimide hydrochloride (EDC·HCl) (Advanced Chemtech), 4-cyano-4-(phenylcarbonothioylthio) pentanoic acid (CPP) (Strem Chemicals), 3-[N-(2-methacroyloyethyl)-N,N-dimethylammonio]propane sulfonate (DMAPS), 2,2'-azobis[2-(2-imidazolin-2-yl)propane]dihydrochloride (VA-044; Wako Chemicals) were used as received. Azobisisobutyronitrile (Sigma-aldrich) was recrystallized twice from methanol. N-isopropylacrylamide (NIPAM) (Sigma-aldrich) was sublimated before use.

Monomer synthesis

Synthesis of 2-ethylsulfanylthiocarbonylsulfanyl-2-methylpropionic Acid (EMP) This procedure was adapted from the previously published procedure[1]. In a 500 mL round-bottom flask ethanethiol (4.5 mL, 62.4 mmol) was added to a suspension of $K_3PO_4 \cdot H_2O$ (12.65 g, 55.0 mmol) in acetone (180 mL) at ambient temperature in a water bath. After stirring for 20 min at ambient temperature, carbon disulfide (9 mL, 149 mmol) was added, and the solution turned yellow. 2-bromoisobutyric acid (8.35 g, 50 mmol) was added after 20 min, and the mixture was stirred at ambient temperature overnight. 1 M HCl (600 mL) was added on an ice bath, and the aqueous phase was extracted with DCM (2 x 300 mL). The combined organic extracts were washed with deionized water (250 mL) and brine (500 mL), dried over Na_2SO_4, and filtered. After evaporation of the solvent, crude product was purified via silica gel chromatography (an Isolera One Biotage system with a KP-Sil cartridge was used with hexane and ethyl acetate as the mobile phase). The fractions containing EMP were collected. The solvent was removed by rotary evaporation, followed by vacuum drying after freezing in liquid nitrogen to give the product as a yellow solid (8.76g, 78%). ^{1}H NMR ($CDCl_3$, δ): 1.33 (*t*, 3H, -$SCH_2C\underline{H}_3$), 1.72 (*s*, 6H, -(C=O)$C(C\underline{H}_3)_2$S-), 3.30 (*q*, 2H, -$SC\underline{H}_2CH_3$).

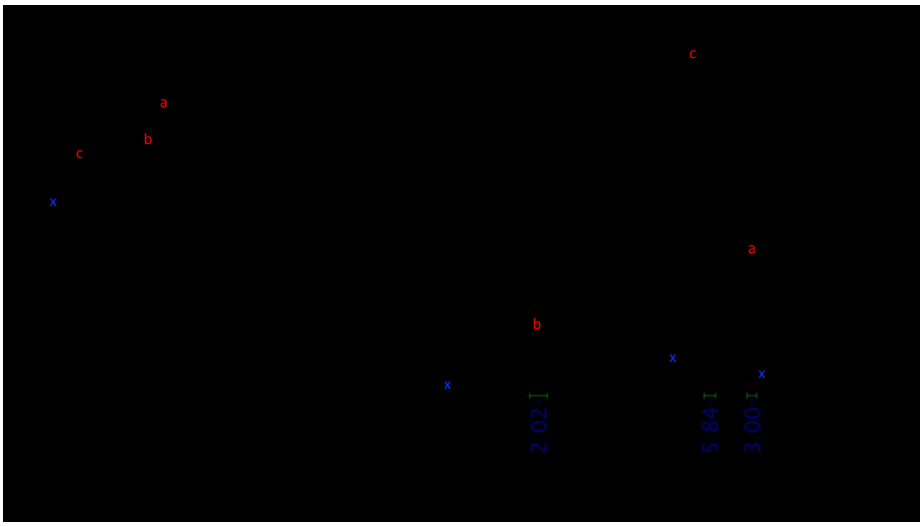


Figure S1 ^{1}H NMR spectrum of EMP.

Synthesis of 2-(3-hydroxypropyl)-3a,4,7,7a-tetrahydro-1*H*-4,7-epoxyisoindole-1,3(2*H*)-dione (pMI-OH) This procedure was adapted from the work of the previously published procedure[2]. In a 500 mL three-neck flask, exo-3,6-Epoxy-1,2,3,6-tetrahydrophthalic anhydride (7.95 g, 47.9 mmol), 3-amino-1-puropanol (3.74 g, 49.8 mmol), and methanol (240 ml) were added. After stirring for 10 min in a water bath at ambient temperature under N_2 flow, trimethylamine (8 mL, 57.4 mmol) was added. The mixture was stirred at 67 °C for 1 day. The solution was reduced to about 100 mL by rotary evaporation, and then ethanol (150 mL) was added. The solvent was removed by rotary evaporation to give white solid. The solid was dissolved in ethanol (250 mL), and then the solution was concentrated to about 50 mL. The solution was held at room temperature overnight to give white crystals. The crystals were filtered, washed with ethanol (50 mL), and dried under vacuum to give the product as white crystals (4.61 g, 43%). ^{1}H NMR ($CDCl_3$, δ): 1.76 (*tt*, 2H, -CH_2(C$\underline{H_2}$)CH_2-)), 2.53 (*brs*, 1H, -O$\underline{H}$), 2.87 (*s*, 2H, -NC(O)C$\underline{H}$-), 3.51 (*t*, 2H, -CH_2(C$\underline{H_2}$)O-), 3.64 (*t*, 2H, -N(C$\underline{H_2}$)CH_2-), 5.27 (*t*, 2H, -C$\underline{H}$(O)-), 6.52 (*t*, 2H, -C$\underline{H}$C$\underline{H}$-).

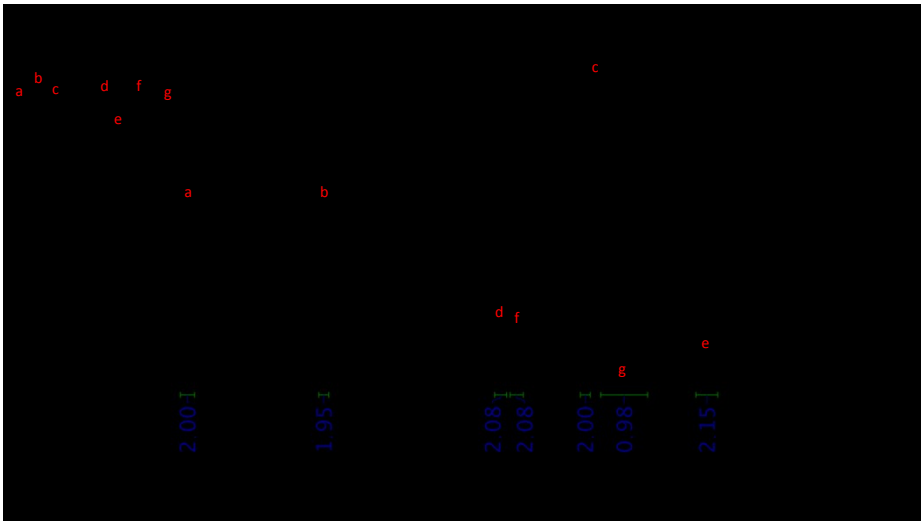


Figure S2 ^{1}H NMR spectrum of pMI-OH.

Synthesis of pMI-EMP In a 100 mL three-neck flask, pMI-OH (2.23g 10.0 mmol), EMP (2.25g, 10.1 mmol), DMAP (0.187g, 1.53 mmol), and anhydrous DCM (20 mL) were added, and the mixture was stirred on an ice bath under N_2 for 30 min. After EDC·HCl (2.89 g, 15.1 mmol) was added to the mixture, it was stirred for 30 min on an ice bath, then stirred for 2 days at ambient temperature under N_2. DCM (10 mL) was added to the mixture, and then the solution was washed with water (3 x 50 mL) and brine (50 mL), dried over $MgSO_4$, filtered, and concentrated to give the crude product as orange oil. The crude was purified via silica gel chromatography (an Isolera One Biotage system with a KP-Sil cartridge was used with hexane and ethyl acetate as eluents.). The fractions containing RAFT1 were collected, and the solvent was removed by rotary evaporation to yield pMI–EMP as yellow solid (3.31 g, 77%) 1H NMR ($CDCl_3$, δ): 1.29 (*t*, 3H, -$SCH_2C\underline{H}_3$), 1.71 (*s*, 6H, -(C=O)C($C\underline{H}_3$)$_2$S-), 1.91 (*tt*, 2H, -$CH_2(C\underline{H}_2)CH_2$-), 2.85 (*s*, 2H, -NC(O)C$\underline{H}$-), 3.26 (*q*, 2H, - $SC\underline{H}_2CH_3$), 3.56 (*t*, 2H, -$N(C\underline{H}_2)CH_2$-), 4.07 (*t*, 2H, -$CH_2(C\underline{H}_2)$O-), 5.26 (*s*, 2H, -C$\underline{H}$(O)-), 6.51 (*s*, 2H, -C$\underline{H}$C$\underline{H}$-).

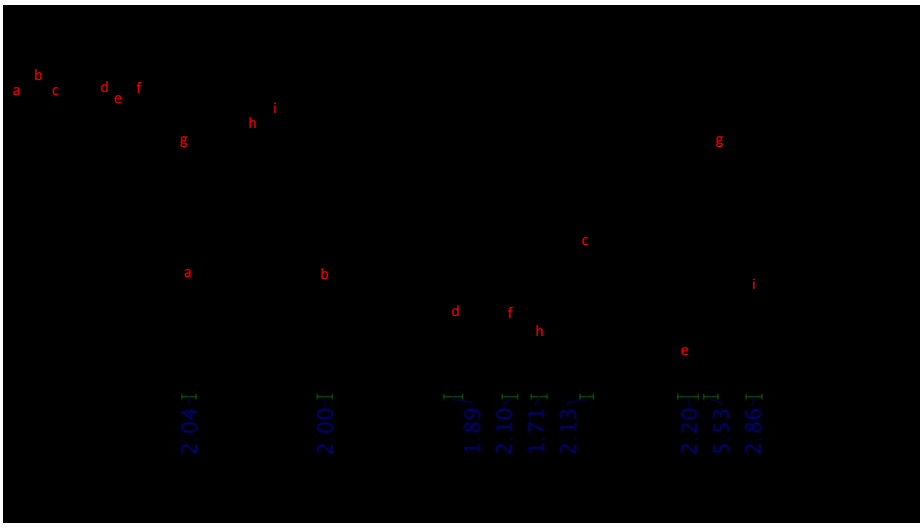


Figure S3 ^{1}H NMR spectrum of pMI-EMP.

Synthesis of 2-(2,3-dihydroxypropyl)-3a,4,7,7a-tetrahydro-1*H*-4,7-epoxyisoindole-1,3(2*H*)-dione (pMI-(OH)$_2$) This procedure was adapted from a previously published procedure[2]. In a 500 mL three-neck flask, exo-3,6-epoxy-1,2,3,6-tetrahydrophthalic anhydride (3.30 g, 19.9 mmol), 3-amino-1,2-propanediol (1.99 g, 21.8 mmol), and methanol (100 mL) were added. After stirring for 10 min in a water bath at ambient temperature under N_2 flow, trimethylamine (2.45 g, 24.2 mmol) was added. The mixture was stirred at 67 °C for 1 day. The solution was concentrated to about 20 mL by rotary evaporation to yield a white solid. The solid was filtered, washed with ethanol (50 mL), and dried under vacuum to give the product as a white solid (3.51 g, 78%) ^{1}H NMR (DMSO-d_6, δ): 2.92 (*d*, 2H, -NC(O)C$\underline{H}$-), 3.26 (*td*, 2H, -CHC$\underline{H}_2$OH), 3.33 (*dd*, 2H, -NC$\underline{H}_2$CH-, overlapped with H_2O), 3,60 – 3.73 (*m*, 1H, -$CH_2(CH_2)(C\underline{H})$OH), 4,57 (*t*, 1H, -$CH_2O\underline{H}$), 4.79 (*d*, 1H, -CHO$\underline{H}$), 5.12 (*t*, 2H, -C$\underline{H}$(O)-), 6.55 (*t*, 2H, -C$\underline{H}C\underline{H}$-).

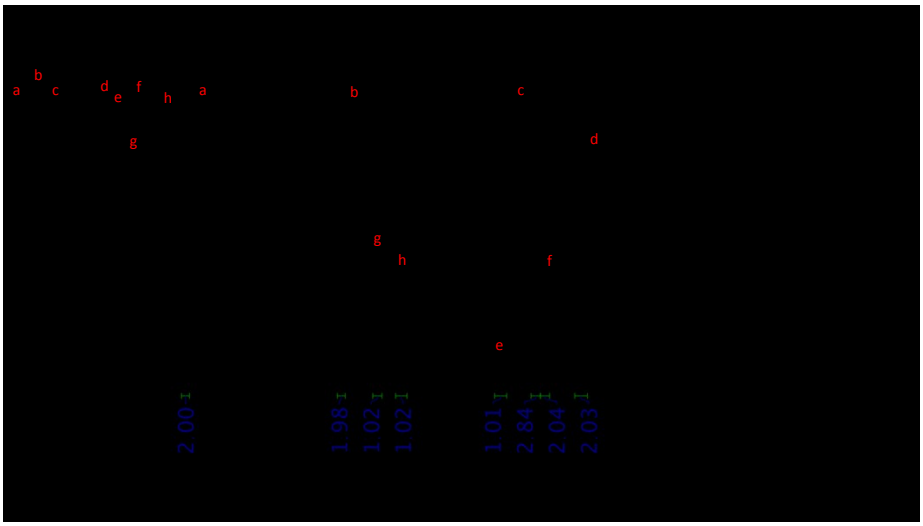


Figure S4 ^{1}H NMR spectrum of pMI-$(OH)_2$.

Synthesis of pMI-$(EMP)_2$ In a 100 mL three-neck flask, pMI-$(OH)_2$ (1.43 g 5.99 mmol), EMP (2.96 g, 13.2 mmol), DMAP (0.290 g, 2.37 mmol), and anhydrous DCM (12 mL) were added, and the mixture was stirred in an ice bath under N_2 for 30 min. After EDC·HCl (4.61 g, 24.0 mmol) was added to the mixture, it was stirred for 30 min on ice, then stirred for 2 days at ambient temperature under N_2. DCM (40 mL) was added to the mixture, and then the solution was washed with water (3 x 50 mL) and brine (50 mL), dried over $MgSO_4$, filtered, and concentrated to give the crude product. The crude product was purified via silica gel chromatography (using an Isolera One Biotage system with a KP-Sil cartridge with hexane and ethyl acetate as eluents.). The fractions containing pMI-$(EMP)_2$ were collected, and the solvent was removed by rotary evaporation to yield pMI-$(EMP)_2$ as sticky yellow solid (2.16 g, 55%) ^{1}H NMR ($CDCl_3$, δ):1.31 (*t*, 6H, -$CH_2C\underline{H}_3$), 1.64 (*s*, 6H, -(C=O)C(C$\underline{H}_3$)$_2$S-), 1.71 (*s*, 3H, -(C=O)C(C$\underline{H}_3$)$_2$S-), 1.73 (*s*, 3H, -(C=O)C(C$\underline{H}_3$)$_2$S-), 2.86 (*dd*, 2H, -NC(O)C$\underline{H}$-), 3.20 – 3.33 (*m*, 4H, -SC$\underline{H}_2$$CH_3$), 3,58 (*dd*, 1H, -CH(C$\underline{H}_2$)O), 3.91 (*dd*, 1H, -CH(C$\underline{H}_2$)O), 4.12 (*dd*, 1H, -N(C$\underline{H}_2$)CH), 4.30 (*dd*, 1H, -N(C$\underline{H}_2$)CH), 5.19-5.26 (overlapped, 3H, -C$\underline{H}$(O)-, -$CH_2(CH_2)$(C$\underline{H}$)O-), 6.50 (s, 2H, -C$\underline{H}$C$\underline{H}$-).

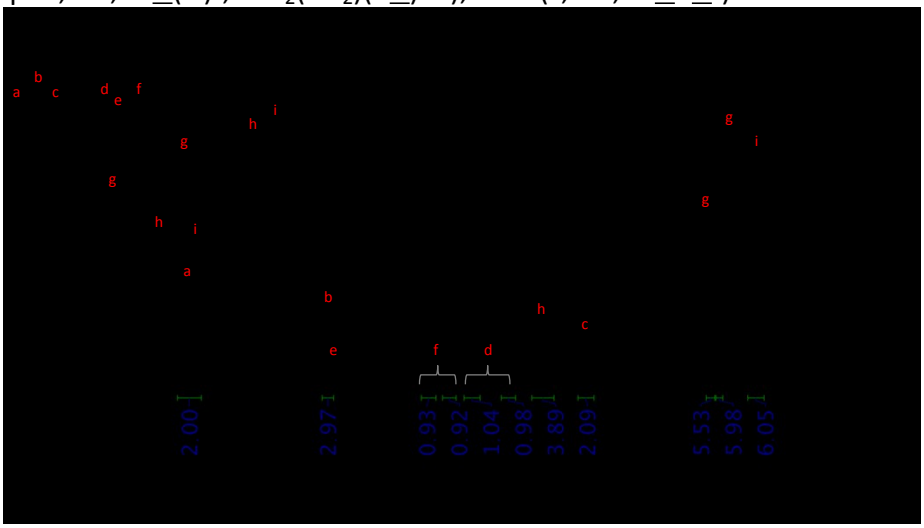


Figure S5 ^{1}H NMR spectrum of pMI-$(EMP)_2$.

Synthesis of pMI-CPP[3] In a 50 mL three-neck flask, pMI-OH (2.25 g, 10.1 mmol), CPP (2.79 g, 9.99 mmol), DMAP (0.156 g, 1.28 mmol), and DCM anhydrous (10 mL) were added, and the mixture was stirred on an ice bath under N_2 for 15 min. After EDC·HCl (2.99 g, 15.0 mmol) was added to the mixture, it was stirred for 15 min on an ice bath, then stirred for 1 day at ambient temperature under N_2. DCM (30 mL) was added to the mixture, and then the solution was washed with water (3 x 40 mL) and brine (40 mL), dried over $MgSO_4$, filtered, and concentrated to give the crude product. The crude product was purified via silica gel chromatography (an Isolera One Biotage system with a KP-Sil cartridge was used with hexane and ethyl acetate as eluents.). The fractions containing CPP-imide were collected, and the solvent was removed by rotary evaporation to yield pMI-CPP as a pink-red oil (3.15 g, 65%) ^{1}H NMR ($CDCl_3$, δ): 1.89-1.98 (*overlapped*, 5H, SC(CN)(CH_2)($C\underline{H_3}$), -$CH_2C\underline{H_2}CH_2$-), 2.27-2.43 (*m*, 4H, -$CH_2C\underline{H_2}$C(O)-, -C(CN)(CH_3)$C\underline{H_2}CH_2$-), 2.84 (*s*, 2H, -NC(O)C$\underline{H}$-), 3.59 (*t*, 2H, -N$C\underline{H_2}CH_2$-), 4.07 (*t*, 2H, -$CH_2C\underline{H_2}$O-), 5.26 (*s*, 2H, -C$\underline{H}$(O)-), 6.50 (*s*, 2H, -C$\underline{H}$C$\underline{H}$-), 7.34 – 7.45 (*m*,2H, Ph), 7.53 – 7.61 (*m*,2H, Ph), 7.88 – 7.95 (*m*, 1H, Ph).

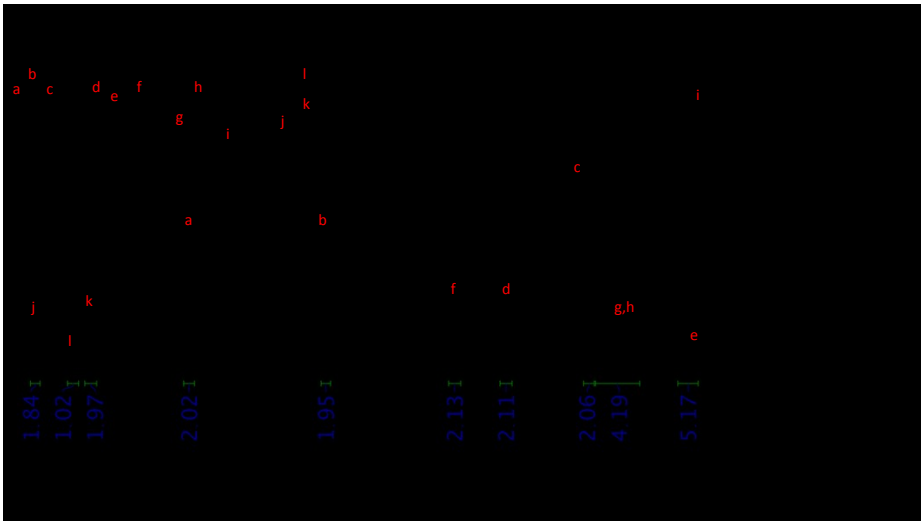


Figure S6 ^{1}H NMR spectrum of Boc-GGG-EMP in $CDCl_3$.

Synthesis of HO-EMP In a 50 mL three-neck flask, EMP (2.24 g, 10.0 mmol), ethylene glycol (2.15 g, 20.1 mmol), DMAP (0.157 g, 1.29 mmol), and anhydrous DCM (10 mL) were added, and the mixture was stirred in an ice bath under N_2 for 30 min. After EDC·Cl (3.84 g, 20.0 mmol) was added to the mixture, it was stirred for 20 min on ice, then stirred for 2 days at ambient temperature under N_2. The solution was washed with water (3 x 30 mL) and brine (30 mL), dried over Na2SO_4, filtered, and concentrated to give the crude product. The crude product was purified via silica gel chromatography (an Isolera One Biotage system with a KP-Sil cartridge was used with hexane/ethyl acetate and Ethyl acetate/DCM as eluents.). The fractions containing HO-EMP were collected, and the solvent was removed by rotary evaporation to yield HO-EMP as an orange oil (1.36 g, including 0.25 equivalent of DCM, 37%) ^{1}H NMR ($CDCl_3$, δ): 1.30 (*t*, 3H, -$SCH_2C\underline{H_3}$), 1.72 (*s*, 6H, -(C=O)C($C\underline{H_3}$)$_2$S-), 3.25 (*q*, 2H, -$SC\underline{H_2}CH_3$), 3.75 – 3.83 (*m*, 2H, -$OC\underline{H_2}CH_2$-), 4.20 – 4.27 (*m*, 2H, -$CH_2C\underline{H_2}$OH).

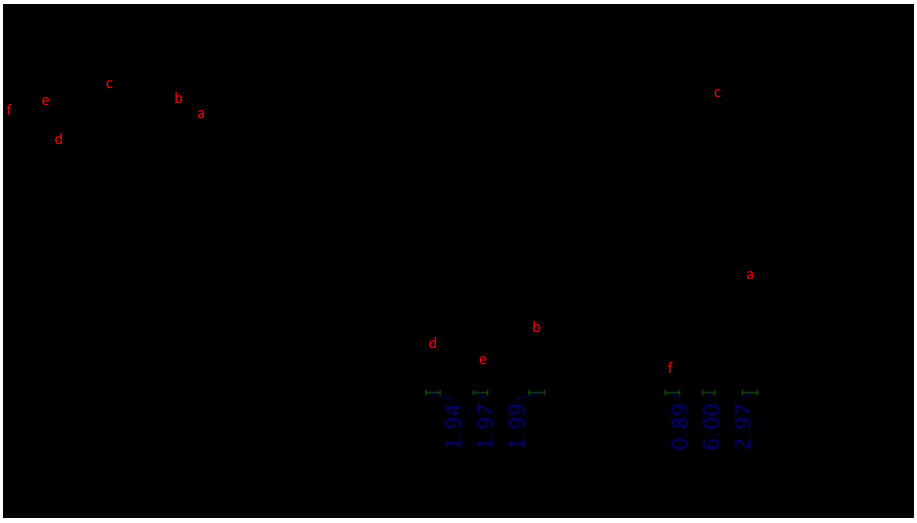


Figure S7 ^{1}H NMR spectrum of HO-EMP in $CDCl_3$.

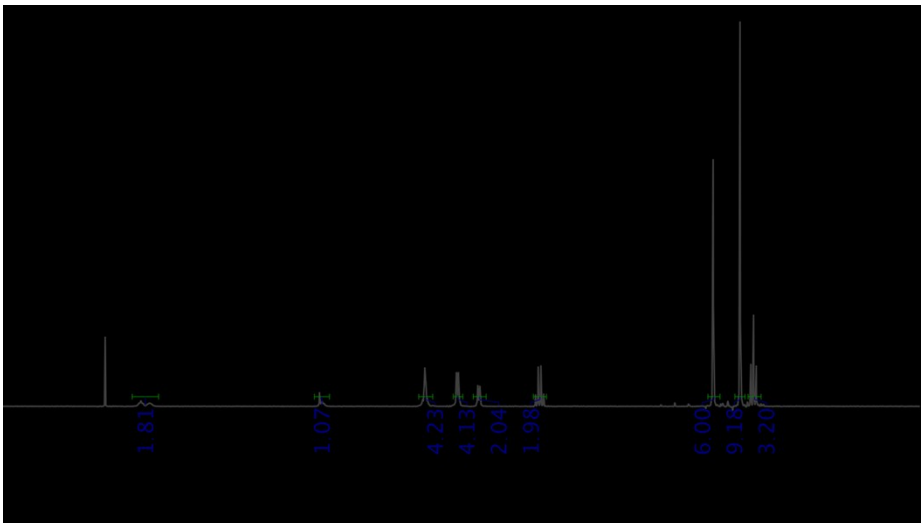


Figure S8 ^{1}H NMR spectrum of Boc-GGG-EMP in $CDCl_3$.

Polymer synthesis

Polymerization (MI-PNIPAM, MI-(PNIPAM)$_2$) poly(N-isopropylacrylamide) (PNIPAM) was synthesized by reversible addition− fragmentation chain transfer (RAFT) polymerization utilizing a maleimide functionalized chain transfer agent (CTA), as reported previously. The CTA, AIBN and NIPAM (sublimated) were dissolved in acetonitrile. After three freeze–pump–thaw cycles, the flask was filled with nitrogen and heated to 60 °C to initiate polymerization. The molecular weight and polydispersity were determined by gel permeation chromatography, and the polymerization was terminated at the appropriate molecular weight by removal of heat and exposure to oxygen. The polymer was then precipitated in cold diethyl ether and dried under vacuum. The maleimide was deprotected by heating to 120 °C under vacuum for 2 h.

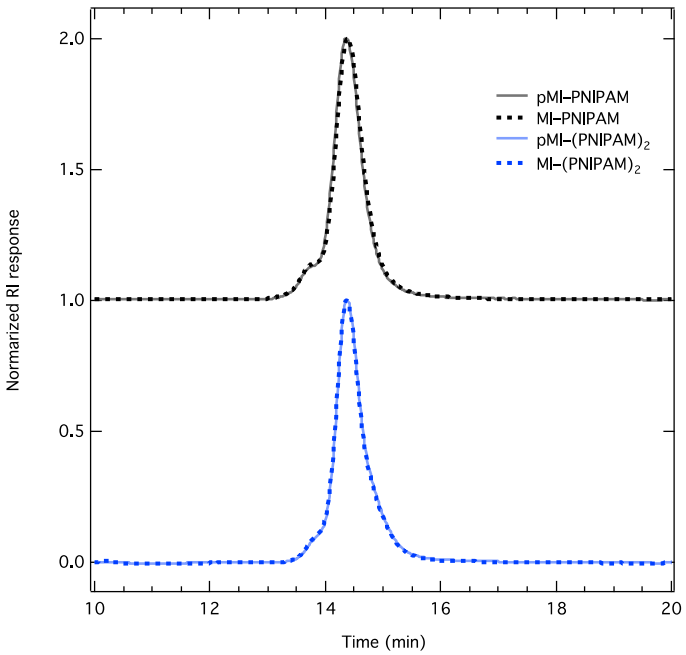


Figure S9 Gel permeation chromatography of PNIPAM for AB and AB_2 type. pMI: before deprotection, MI: after deprotection, PNIPAM: 1-arm PNIPAM, $(PNIPAM)_2$: 2-arm PNIPAM. pMI–PNIPAM_29K: M_n = 29,340 g/mol; M_w = 33,330 g/mol; $Đ$ = 1.14, MI–PNIPAM_29K: M_n = 29,080 g/mol; M_w = 33,740 g/mol; $Đ$ = 1.16. pMI–$(PNIPAM)_2$_29K: M_n = 27,830 g/mol; M_w = 32,450 g/mol; $Đ$ = 1.17, MI–$(PNIPAM)_2$_29K: M_n = 28,610 g/mol; M_w = 32,750 g/mol; $Đ$ = 1.15.

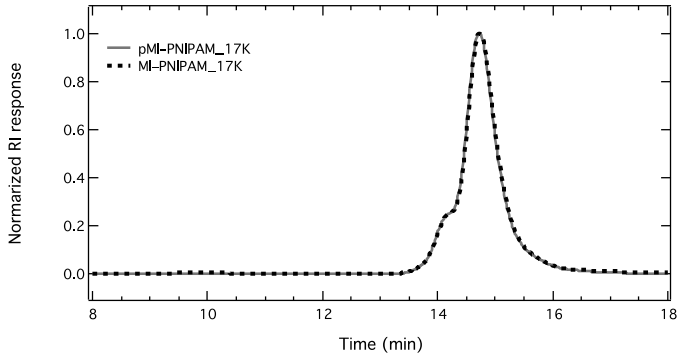


Figure S10 Gel permeation chromatography of PNIPAM for ABA type. pMI–PNIPAM_17K: M_n = 17,300 g/mol; M_w = 18,710 g/mol; $Đ$ = 1.08, MI–PNIPAM_17K: M_n = 17,430 g/mol; M_w = 18,670 g/mol; $Đ$ = 1.07.

Polymerization (GGG–PNIPAM) NIPAM, Boc-GGG-EMP, and AIBN were dissolved in acetonitrile (NIPAM : Boc-GGG-EMP : AIBN = 140 : 1 : 0.2). After three freeze–pump–thaw cycles, the flask was filled with nitrogen and heated to 60 °C to initiate polymerization. The molecular weight and polydispersity were determined by gel permeation chromatography, and the polymerization was terminated at appropriate molecular weight by removal of heat and exposure to oxygen. The polymer (Boc–GGG–PNIPAM) was then precipitated in cold diethyl ether and dried under vacuum. The Boc protecting group was removed by trifluoroacetic acid. To deprotect, Boc–GGG–

PNIPAM (8 g) was dissolved in 80 mL of anhydrous CH_2Cl_2, and 40 mL of trifluoroacetic acid was then added. The solution was stirred at room temperature overnight. The solution was poured into an excess of cold ethyl ether to yield a light yellow liquid product at the bottom of the flask. The solvent was removed by decantation, and the product was dried under vacuum. The product (GGG–PNIPAM) was dissolved to acetonitrile and the polymer was precipitated in cold diethyl ether (twice), followed by filtered and dried under vacuum at room temperature (6.26g, 78%).

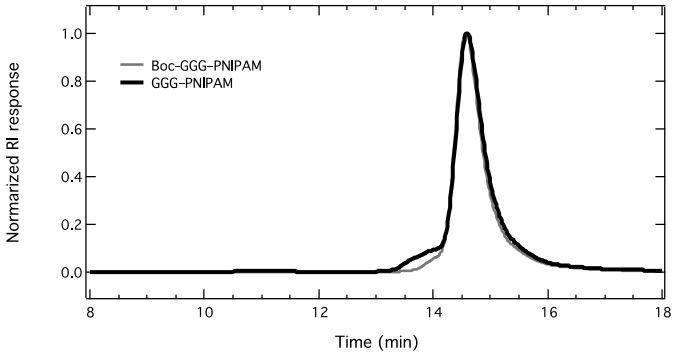


Figure S11 Gel permeation chromatography of PNIPAM for ABA and ABC type. Boc–GGG–PNIPAM: M_n = 17,780 g/mol; M_w = 18,070 g/mol; $Đ$ = 1.02, GGG–PNIPAM: M_n = 17,760 g/mol; M_w = 18,670 g/mol; $Đ$ = 1.05.

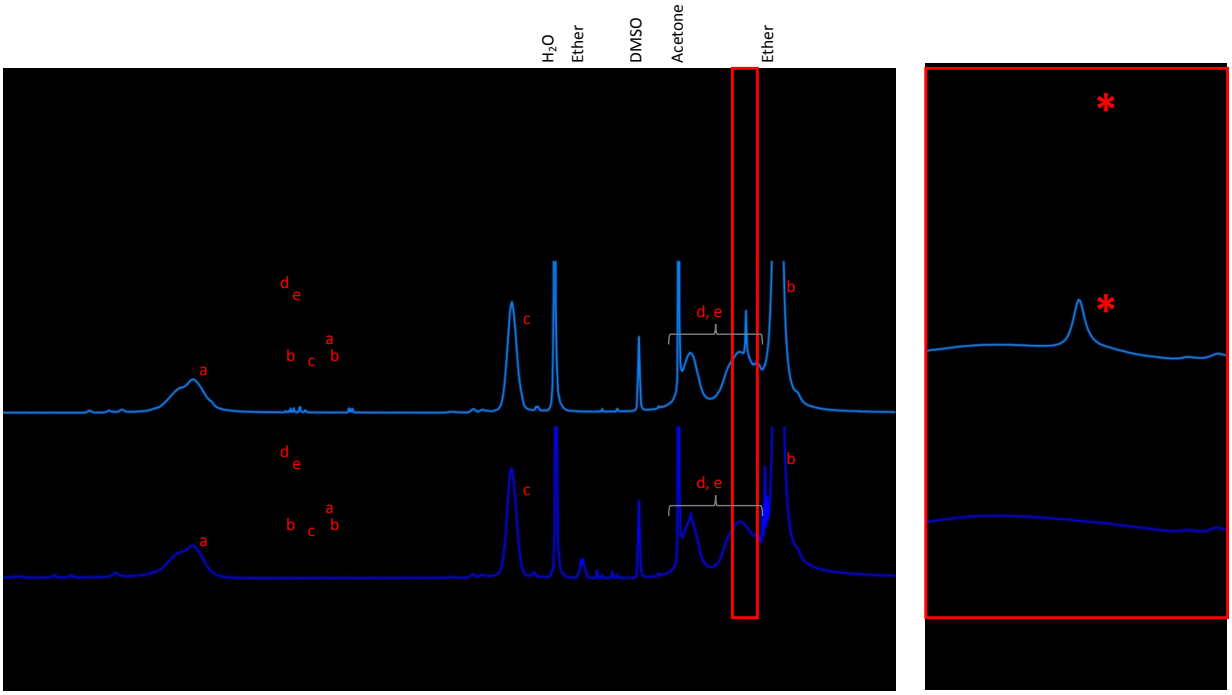


Figure S12 ^{1}H NM spectrum of Boc-GGG-EMP (top), Boc–GGG–PNIPAM (midle), GGG–PNIPAM (bottom). Boc peak disappeared after deprotection (see enlarged view, right).

Polymerization (MI-PDMAPS) Poly (3-[N-(2- methacroyloyethyl)-N,N-dimethylammonio]propane sulfonate)) (PDMAPS) was synthesized by reversible addition−fragmentation chain transfer (RAFT) polymerization utilizing a maleimide-functionalized chain transfer agent (CTA), as reported previously. DMAPS, a chain transfer agent (pMI-CPP), and initiator VA-044 were dissolved in trifluoroethanol (DMAPS : pMI-CPP : VA-044 = 120 : 1 : 0.5). After three freeze–pump–thaw cycles, the flask was filled with nitrogen and heated to 40 °C to initiate polymerization. The molecular weight and polydispersity were determined by gel permeation chromatography, and the polymerization was terminated at appropriate molecular weight by cooling in an ice bath and exposure to oxygen. The polymer was precipitated in methanol (three times) and dried under vacuum.

To remove the thiocarbonyl end group, the polymer was dissolved in trifluoroethanol, and 40 equivalents of AIBN were added. After three freeze–pump–thaw cycles, the flask was filled with nitrogen and heated to 80 °C for 2.5 h. After the reaction with AIBN, the polymer was precipitated in acetone. The polymer was isolated by filtration and dried under vacuum. The polymer was dissolved in TFE, precipitated in methanol, and dried under vacuum (twice).

For deprotection of the maleimide, the polymer was heated at 150 °C for 2 hours under vacuum.

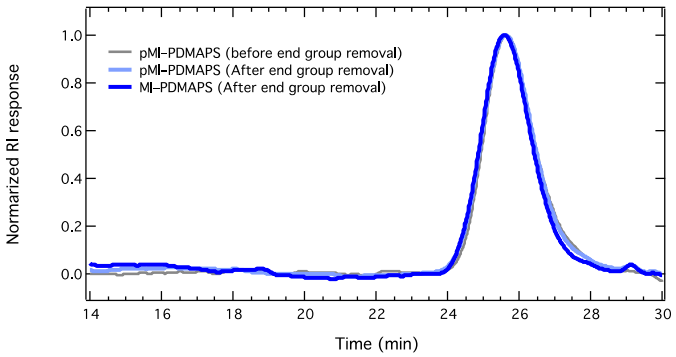
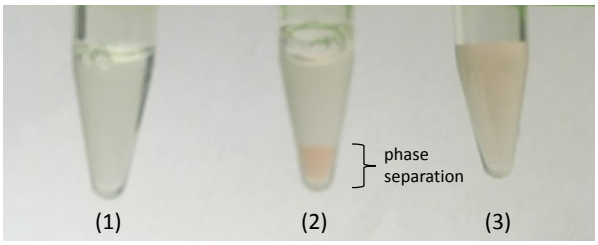


Figure S13 Gel permeation chromatography of PDMAPS used in the construction of ABC type copolymers. pMI–PDMAPS (before end group removal): M_n = 17,030 g/mol; M_w = 21,570 g/mol; $Đ$ = 1.27, pMI–PDMAPS (After end group removal): M_n = 18,540 g/mol; M_w = 23,500 g/mol; $Đ$ = 1.27, MI–PDMAPS(after end group removal and deprotection of maleimide): M_n = 19,420 g/mol; M_w = 25,200 g/mol; $Đ$ = 1.30.



(1) 200 µL of PNIPAM (100 mg/mL)
(2) a mixture of 100 µL of PNIPAM (100 mg/mL) and 100 µL of PDMAPS (100 mg/mL)
(3) 200 µL of PDMAPS (100 mg/mL)

Figure S14 Photograph of aqueous solutions of (1) PNIPAM, (2) mixture of PNIPAM and PDMAPS, and (3) PDMAPS.

Cloning and Protein Expression

mCherryS131C mCherryS131C was expressed and purified according to a previously described method[1].

mCherryS131C–LPETGG–His$_6$ The plasmid for mCherryS131C–LPETGG–His$_6$ was purchased from GenScript (Piscataway, NJ 08854USA). DNA sequence of mCherryS131C–LPETGG–His$_6$ was inserted to pET22b-(+) by using NdeI/HindIII as cloning site.

DNA sequence (cloning sites were underlined)

CATATGGTGAGCAAGGGCGAGGAGGATAACATGGCCATCATCAAAGAATTTATGCGTTTCAAA
GTGCACATGGAAGGTAGCGTTAATGGCCACGAATTTGAAATTGAAGGCGAAGGTGAAGGTCGT
CCGTATGAAGGTACCCAGACGGCAAAACTGAAAGTGACCAAAGGCGGTCCGCTGCCGTTTGCC
TGGGATATTCTGTCTCCGCAGTTCATGTATGGTAGTAAAGCGTACGTTAAACATCCGGCCGATA
TCCCGGATTATCTGAAACTGTCTTTTCCGGAAGGCTTCAAATGGGAACGTGTGATGAACTTCGA
AGATGGCGGTGTGGTTACCGTTACGCAGGATAGCTCTCTGCAGGATGGTGAATTTATCTACAAA
GTGAAACTGCGCGGTACCAATTTCCCGTGCGATGGCCCGGTTATGCAGAAGAAAACCATGGGC
TGGGAAGCCAGTAGCGAACGTATGTACCCGGAAGATGGTGCACTGAAAGGCGAAATCAAACA
GCGCCTGAAACTGAAAGATGGCGGTCACTATGATGCAGAAGTGAAAACCACGTACAAAGCGAA
AAAACCGGTGCAGCTGCCGGGTGCATACAACGTTAACATCAAACTGGATATCACCAGCCATAAC
GAAGATTATACGATCGTTGAACAGTACGAACGTGCAGAAGGTCGTCACTCTACCGGTGGTATG
GATGAACTGTACAAAGGTGGCGGTGGCAGCCTCCCGGAAACCGGTGGCCATCACCACCACCAT
CACTAAAAGCTT

Amino Acid Sequence.

MVSKGEEDNMAIIKEFMRFKVHMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFAWDI
LSPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGEFIYKVKLRG
TNFPCDGPVMQKKTMGWEASSERMYPEDGALKGEIKQRLKLKDGGHYDAEVKTTYKAKKPVQLP
GAYNVNIKLDITSHNEDYTIVEQYERAEGRHSTGGMDELYKGGGGSLPETGGHHHHHH*

Expression of mCherryS131C–LPETGG–His$_6$.

The protein was expressed in the *Escherichia coli* strain BL21(DE3) (product #C2527I, New England Biolabs). The cells were grown in Lysogeny Broth (4 x 1 L) at 37 °C until reaching OD_{600} =

0.8-1.0, and expression was induced by adding 1 mM isopropyl β-D- 1-thiogalactopyranoside and lowering the temperature to 30 °C. The cells were harvested 8 hours after induction and resuspended in 50 mL lysis buffer (50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole, 10 mM β-mercaptoethanol (BME), adjusted to pH 8.0 with NaOH) per 1 L of cell culture. The cell suspensions were then lysed by incubating with 1 mg/mL lysozyme for 30 min followed by sonication. The resulting lysate was clarified by centrifugation, and the protein was purified using Ni-NTA metal affinity chromatography. The bound resin was washed with 8 column volumes of wash buffer (50 mM NaH_2PO_4, 300 mM NaCl, 20 mM imidazole, 10 mM BME, adjusted to pH 8.0) and eluted with 4 column volumes of elution buffer (50 mM NaH_2PO_4, 300 mM NaCl, 250 mM imidazole, 10 mM BME, adjusted to pH 8.0). Elution fractions containing the purified mCS131C-L-LPETG-His were dialyzed into 20 mM Tris-HCl buffer (pH 8.0), and purity was confirmed by denaturing polyacrylamide gel electrophoresis (SDS-PAGE, Figure S19). Yield was determined by measuring A586 (extinction coefficient 72,000 M^{-1} cm^{-1}) on a Varian Cary 50 Bio UV/vis spectrophotometer. Typical yields after purification were around 200 mg/L culture.

Sortase A The plasmid for sortase A pentamutant (eSrtA) in pET29 was a gift from David Liu (Addgene plasmid # 75144). Sortase A was expressed and purified according to a previously described method[4]. The cells were grown in Lysogeny Broth (4 x 1 L) with 50 µg/mL kanamycin at 37 °C until reaching OD_{600} = 0.5-0.8, and expression was induced by adding 0.4 mM isopropyl β-D- 1-thiogalactopyranoside and lowering the temperature to 30 °C. The cells were harvested 3 hours after induction and resuspended in 200 mL Tris-HCl buffer (50 mM Tris, 300mM NaCl, pH8). 200 µL of 1M MgCl2, 200 µL of DNAseI (2000 unit/mL), 67.6 µL of 5 mg/mL aprotinin, 24 µL of 10 mM leupetin, and 34.8 mg of phenylmethanesulfonyl fluoride were added to the suspended solution.

The cell suspension was lysed by incubating for 60 min at 4 °C followed by sonication. The resulting lysate was clarified by centrifugation, and the protein was purified using Ni-NTA metal affinity chromatography. The bound resin was washed with wash buffer (50 mM NaH_2PO_4, 300 mM NaCl, 20 mM imidazole, 10 mM BME, adjusted to pH 8.0) and eluted with elution buffer (50 mM NaH_2PO_4, 300 mM NaCl, 250 mM imidazole, 10 mM BME, adjusted to pH 8.0). Elution fractions containing the purified Sortase A were dialyzed into Tris-HCl buffer (25 mM Tris, 150 mM NaCl, pH7.5), and purity was confirmed by denaturing polyacrylamide gel electrophoresis (SDS-PAGE, Figure S15). Enzyme concentration was calculated from the measured A_{280} using the published extinction coefficient of 17,420 M^{-1} cm^{-1}.

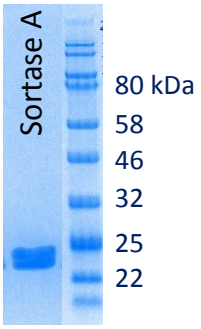


Figure S15. SDS-PAGE of Sortase A.

Bioconjugation

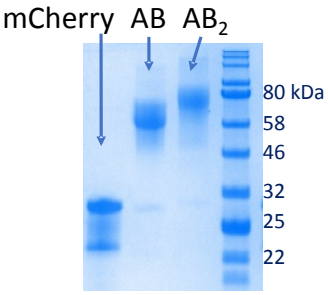


Figure S16. SDS-PAGE of mCherryS131C (mCherry), mCherry–PNIPAM (AB), and mCherry–$(PNIPAM)_2$ (AB_2).

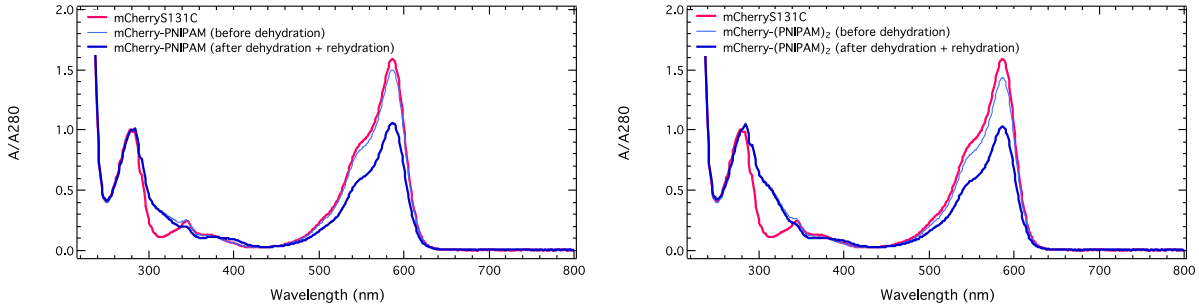


Figure S17. UV-Vis spectrum of Left: mCherryS131C (mCherry) and mCherry–PNIPAM (before dehydration and after dehydration), and Right: mCherryS131C (mCherry) and mCherry–$(PNIPAM)_2$ (before dehydration and after dehydration + rehydration) The signal is normalized using an estimated absorbance of mCherry at 280 nm. Plot of A586/A280 show that 94% of mCherry activity is preserved in mCherry–PNIAPM. The activity of mCherry in mCherry–$(PNIAPM)_2$ is estimated to be 90% of the original mCherry activity. mCherry–PNIAPM and mCherry–$(PNIAPM)_2$ retain 71% and 72% of activity of conjugates (before dehydration) after dehydration and rehydration.

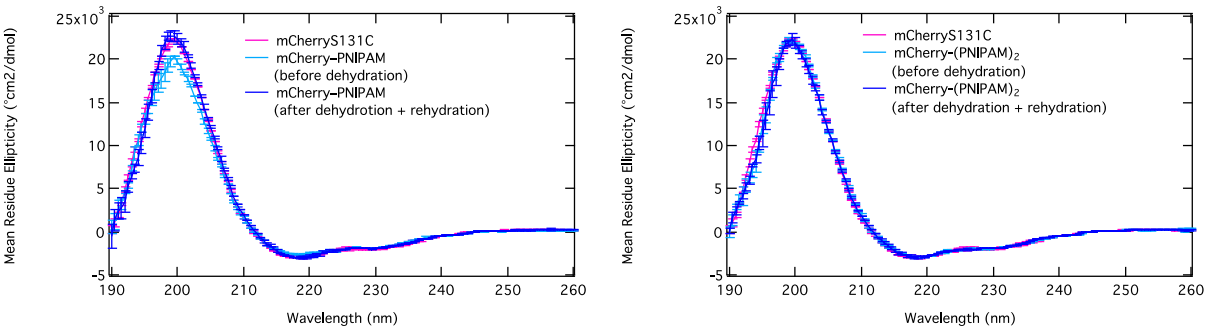


Figure S18. CD spectrum of Left: mCherryS131C (mCherry) and mCherry–PNIPAM (before dehydration and after dehydration), and Right: mCherryS131C (mCherry) and mCherry–$(PNIPAM)_2$ (before dehydration and after dehydration + rehydration).

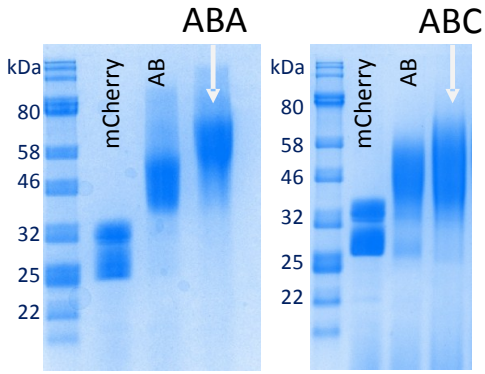


Figure S19. SDS-PAGE of Left: mCherryS131C–LPETGG–His (mCherry), PNIPAM–mCherry–LPETG–His (AB), and PNIPAM–mCherry–PNIPAM(ABA), Right: mCherryS131C–LPETGG–His (mCherry), PDMAPS–mCherry–LPETG–His (AB), and PDMAPS–mCherry–PNIPAM(ABA).

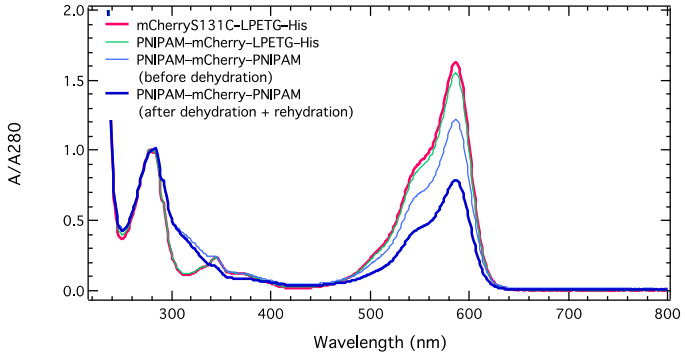


Figure S20. UV-Vis spectrum of mCherryS131C–LPETGG–His, PNIPAM–mCherry–PNIPAM (before dehydration), and PNIPAM–mCherry–PNIPAM (after dehydration + rehydration). The signal is normalized using an estimated absorbance of mCherryS131C-LPETG-His at 280 nm. Plot of A586/A280 show that 95% of mCherry function is preserved in PNIPAM–mCherryS131C-LPETG-His. The function of mCherry in PNIPAM–mCherry–PNIAPM is estimated to be 74% of the original mCherry function. PNIPAM–mCherry–PNIAPM (after dehydration + rehydration) retains 65% of the function of PNIPAM–mCherry–PNIAPM (before dehydration).

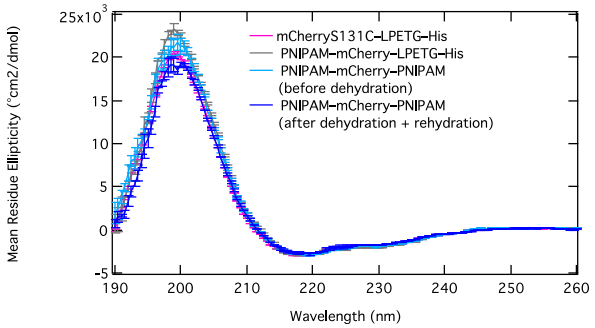


Figure S21. CD spectrum of mCherryS131C–LPETGG–His, PNIPAM–mCherry–PNIPAM (before dehydration), and PNIPAM–mCherry–PNIPAM (after dehydration + rehydration).

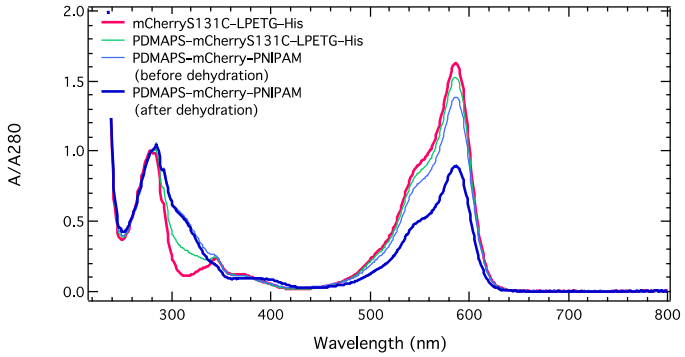


Figure S22. UV-Vis spectrum of mCherryS131C–LPETGG–His, PDMAPS–mCherry–PNIPAM (before dehydration), and PDMAPS–mCherry–PNIPAM (after dehydration + rehydration). The signal is normalized using an estimated absorbance of mCherryS131C-LPETG-His at 280 nm. Plot of A586/A280 show that 94% of mCherry function is preserved in PDMAPS–mCherryS131C-LPETG-His. The function of mCherry in PDMAPS–mCherry–PNIAPM is estimated to be 85% of the original mCherry function. PDMAPS–mCherry–PNIAPM (after dehydration + rehydration) retain 65% of the function of PDMAPS–mCherry–PNIAPM (before dehydration).

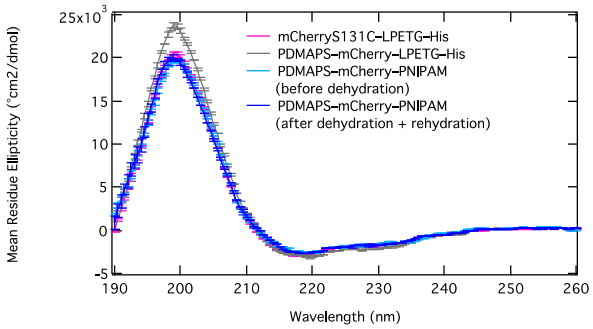


Figure S23. UV-Vis spectrum of mCherryS131C–LPETGG–His, PDMAPS–mCherry–PNIPAM (before dehydration), and PDMAPS–mCherry–PNIPAM (after dehydration + rehydration).

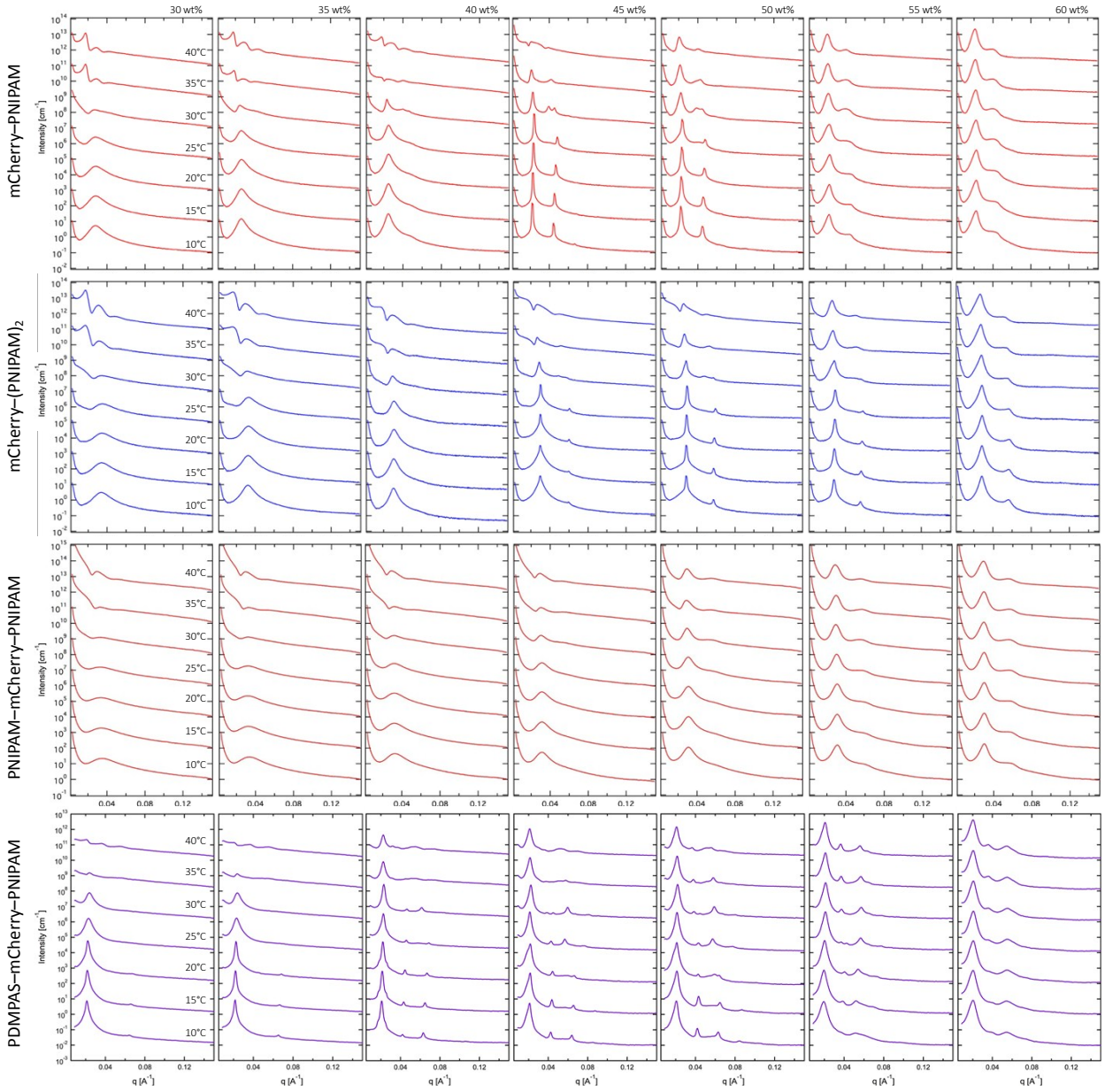


Figure S24. SAXS intensities for each conjugate as a function of temperature and solution concentration.

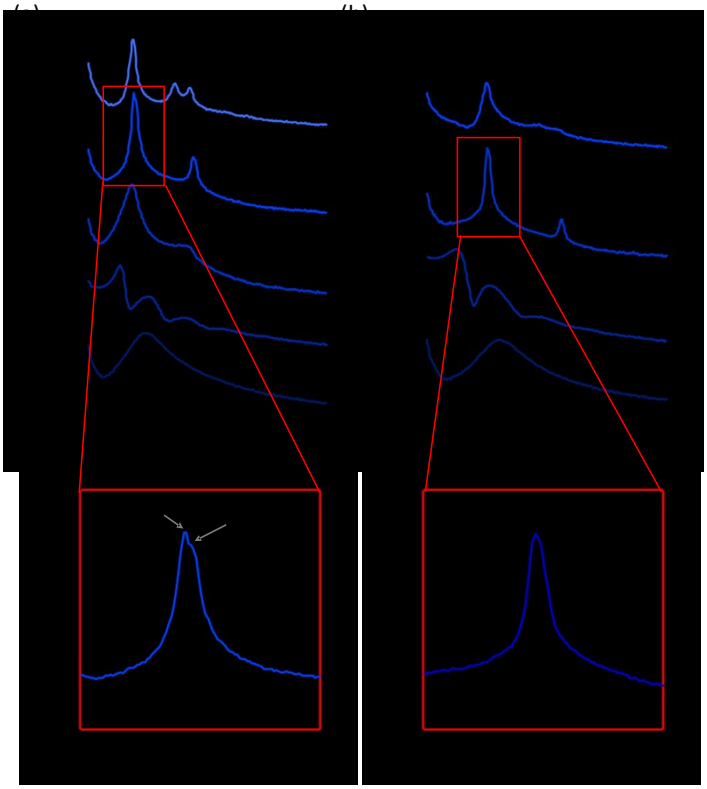


Figure S25. Enlarged view of representative SAXS curves for mCherry–PNIPAM (a) and mCherry–$(PNIPAM)_2$ (b). The same splitting of the primary peak observed at 50% and 20 °C is also observed in the higher order peaks.

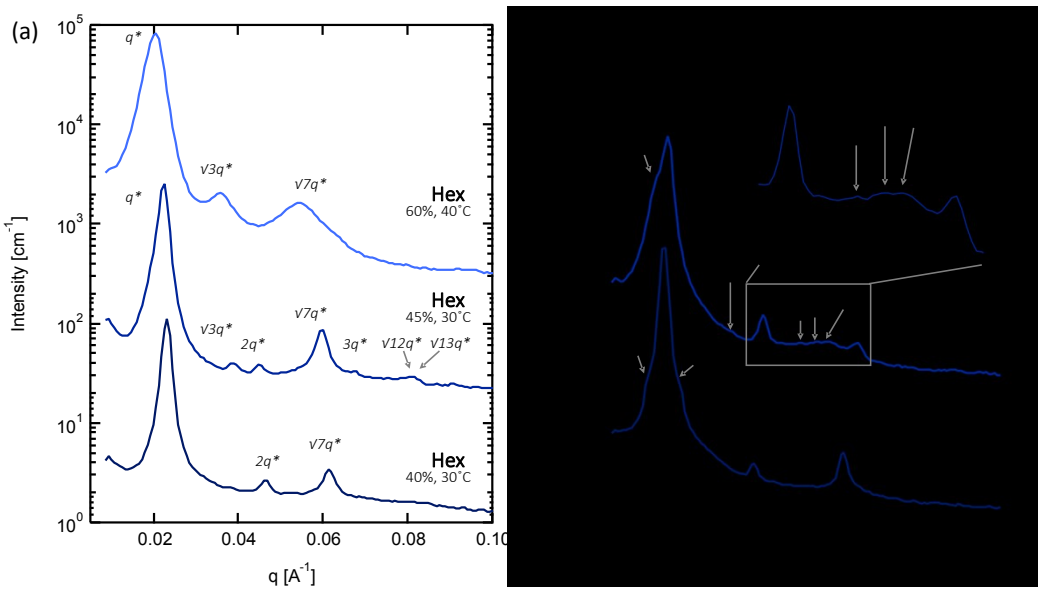


Figure S26. Enlarged view of representative SAXS curves for PDMAPS–mCherry–PNIPAM: (a) hexagonal and (b) perforated lamellar phase.

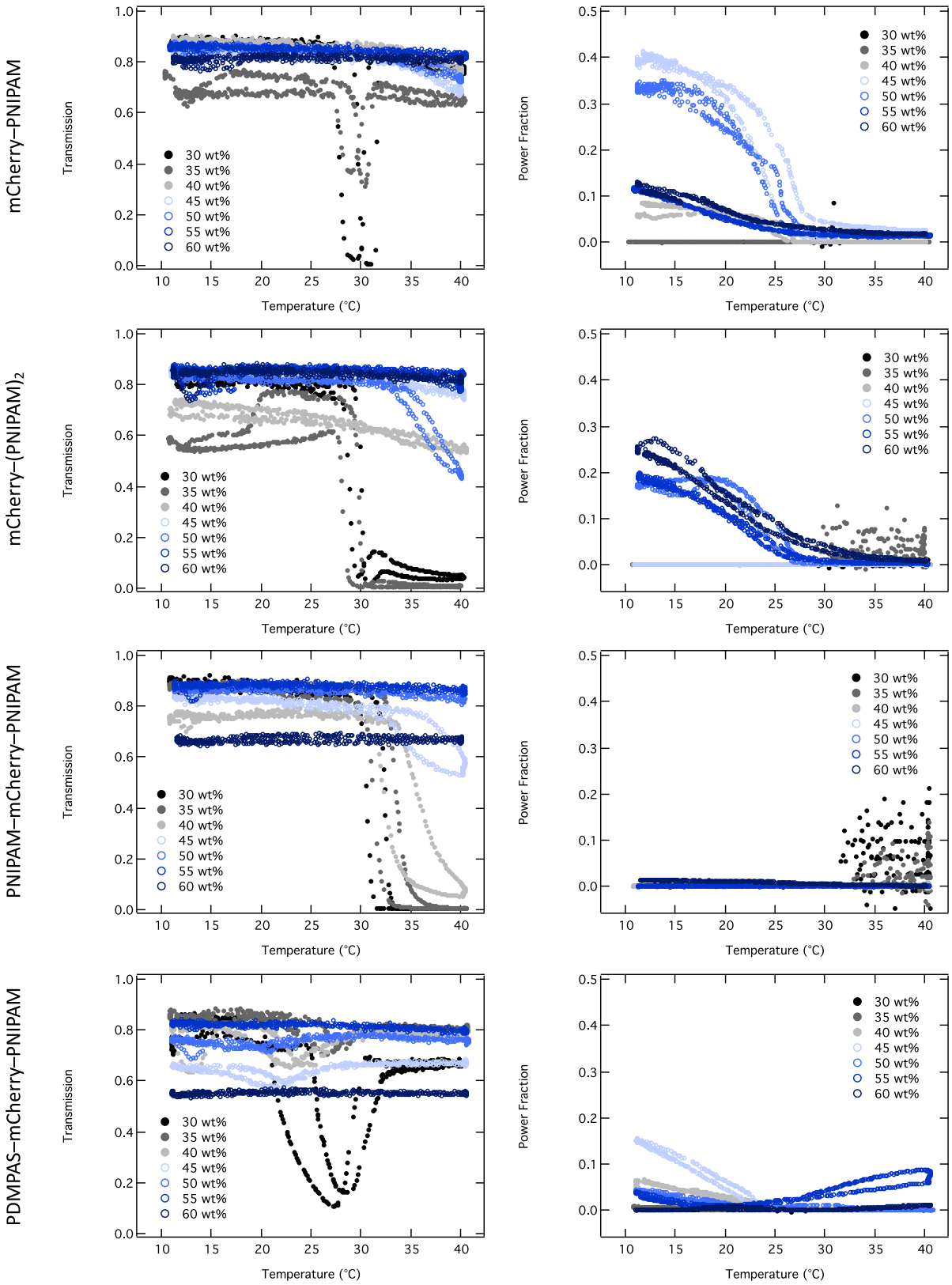


Figure S27. Transmission at λ = 662 nm and birefringence intensities for each conjugate as a function of temperature and solution concentration.

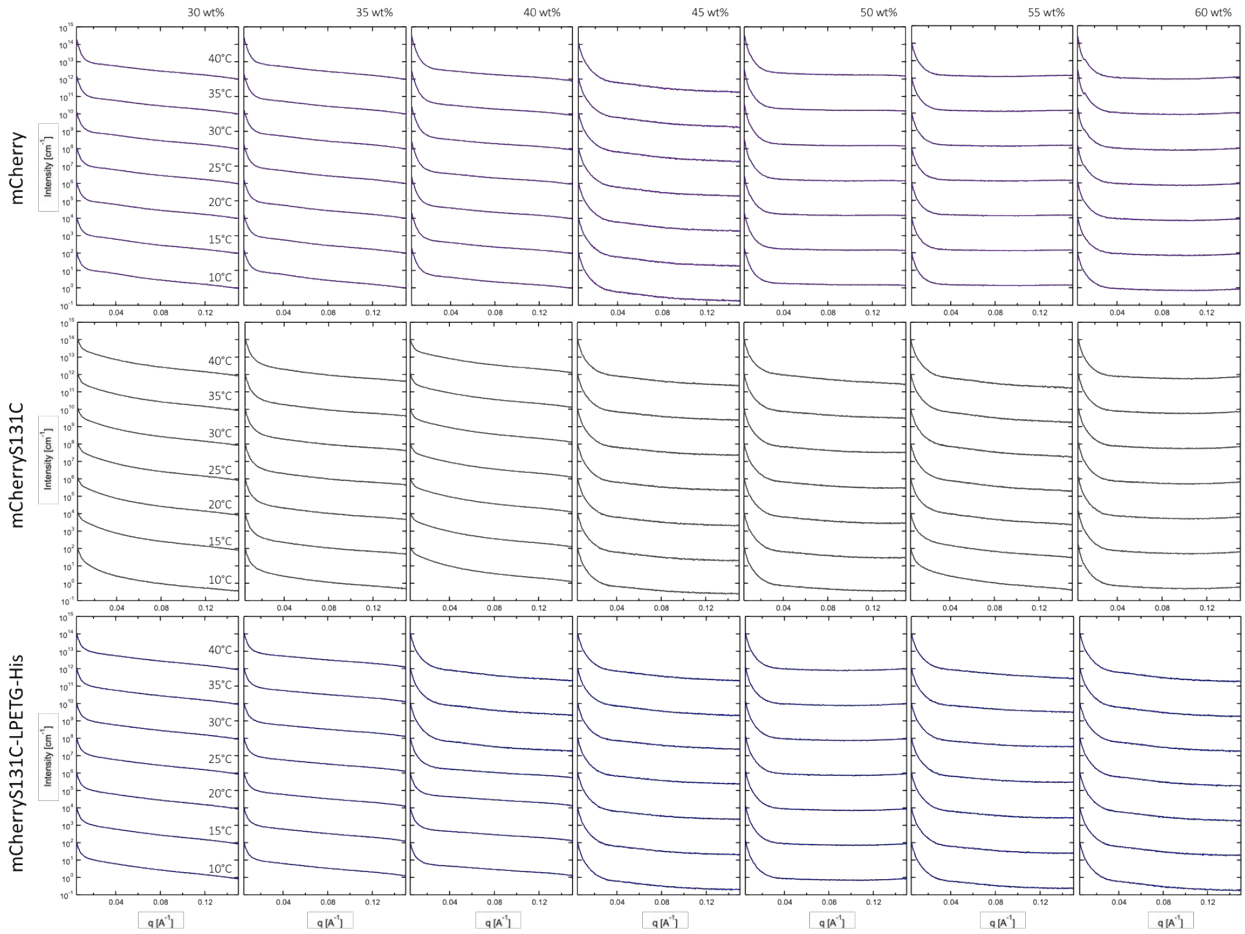


Figure S28. SAXS intensities for three unmodified mCherry as a function of temperature and solution concentration. All three mCherry show no ordered phase.

Domain Spacing Scaling Analysis

Solid state domain spacing d for a copolymer containing a rigid block can be calculated as $d = Na\{Olsen, 2007\ \#84\}$,[5] where N is the number of volumetric repeat units in the block copolymer and a is the statistical segment length of rigid block. The total number volumetric repeat units in the block can thus be represented as $N = N_{protein} + N_{polymer}$. This can be rewritten in terms of

$$d = N_{protein}a\left(1 + \frac{N_{polymer}}{N_{protein}}\right) = N_{protein}a\left(\frac{N_{protein} + N_{polymer}}{N_{protein}}\right) = N_{protein}a\left(\frac{1}{1 - \phi_{polymer}}\right)$$

The polymer coil fraction ϕ can the calculated as below;

$$\phi_{polymer} = \frac{\frac{MW_{polymer}}{\rho_{polymer}}}{\frac{MW_{polymer}}{\rho_{polymer}} + \frac{MW_{protein}}{\rho_{protein}}}$$

For the triblock copolymer, this can be rewritten as follows;

$$\phi_{polymer} = \frac{\frac{MW_{polymer1}}{\rho_{polymer1}} + \frac{MW_{polymer2}}{\rho_{polymer2}}}{\frac{MW_{polymer1}}{\rho_{polymer1}} + \frac{MW_{polymer2}}{\rho_{polymer2}} + \frac{MW_{protein}}{\rho_{protein}}}$$

$$\phi_{polymer1} = \frac{\frac{MW_{polymer1}}{\rho_{polymer1}}}{\frac{MW_{polymer1}}{\rho_{polymer1}} + \frac{MW_{polymer2}}{\rho_{polymer2}} + \frac{MW_{protein}}{\rho_{protein}}}$$

$$\phi_{polymer2} = \frac{\frac{MW_{polymer2}}{\rho_{polymer2}}}{\frac{MW_{polymer1}}{\rho_{polymer1}} + \frac{MW_{polymer2}}{\rho_{polymer2}} + \frac{MW_{protein}}{\rho_{protein}}}$$

In Table1, $\phi_{maleimide}$, $\phi_{triglycine}$, ϕ_{total} represent $\phi_{polymer1}$, $\phi_{polymer2}$, $\phi_{polymer}$, respectively.

To calculate the polymer coil fraction ϕ, the following densities are used for this calculation; $\rho_{PNIPAM} = 1.05$, $\rho_{PDMAPS} = 1.37$, $\rho_{mCherry} = 1.35$. From the crystal structure of mCherry, the β-barrel length of 4.4 nm was used as $N_{protein}a$.

This results in the following domain spacings:
$d_{mCherry\text{–}PNIPAM} = 11.17\ nm$
$d_{mCherry\text{–}(PNIPAM)_2} = 10.97\ nm$
$d_{PNIPAM\text{–}mCherry\text{–}PNIPAM} = 11.95\ nm$
$d_{PDMAPS\text{–}mCherry\text{–}PNIPAM} = 11.73nm$

To account for swelling of the block copolymer domain in concentrated solution, we use the following approximation to estimate the swollen domain spacing d'. [6]
$d' = d(1/z)^{1/3}$

where z is the weight fraction of the bioconjugate. This result in following domain spacing:
$d'_{mCherry\text{–}PNIPAM,\ 50wt\%} = 14.07\ nm$
$d'_{mCherry\text{–}(PNIPAM)_2,\ 50wt\%} = 13.82\ nm$
$d'_{PNIPAM\text{–}mCherry\text{–}PNIPAM,\ 55wt\%} = 14.58\ nm$
$d'_{PDMAPS\text{–}mCherry\text{–}PNIPAM,\ 50wt\%} = 14.78nm$

The swollen diameter ⌀' can be estimated using approximate β-barrel diameter of 2.5 nm.
$⌀' = ⌀(1/z)^{1/3}$

$⌀'_{50wt\%} = 3.15\ nm$

The cross-section area A corresponding to one bioconjugate can be estimated[7]
$$A = \frac{MW_{polymer}}{\rho_{polymer'} N_A d_1 \phi_{polymer}}$$

where N_A is the Avogadro constant and $d_1$is the domains spacing corresponding one bioconjugate. In the case of bilayer lamellar morphology, $d_1 = d_{SAXS}/2$, where d_{SAXS} is domain spacing calculated from SAXS curves: $d_{SAXS,\ mCherry\text{–}PNIPAM,\ 50wt\%,\ 10°C} = 27.4\ nm$ and $d_{SAXS,\ mCherry\text{–}(PNIPAM)_2,\ 50wt\%,\ 10°C} = 21.7\ nm$. To calculate the cross-section area in concentrated solution, following approximation was used.
$\rho_{polymer'} = z\rho_{polymer} + (1 - z)\rho_{water}$

For the diblock copolymers, this results in following cross-section area:
$A_{mCherry\text{–}PNIPAM,\ 50wt\%} = 6.58\ nm^2$
$A_{mCherry\text{–}(PNIPAM)_2,\ 50wt\%} = 8.16\ nm^2$

These cross-section area A suggest swollen diameter ⌀
$⌀'_{SAXS,\ mCherry\text{–}PNIPAM,\ 50wt\%,10°C} = 2.90\ nm$
$⌀'_{SAXS,\ mCherry\text{–}(PNIPAM)_2,\ 50wt\%,10°C} = 3.22\ nm$

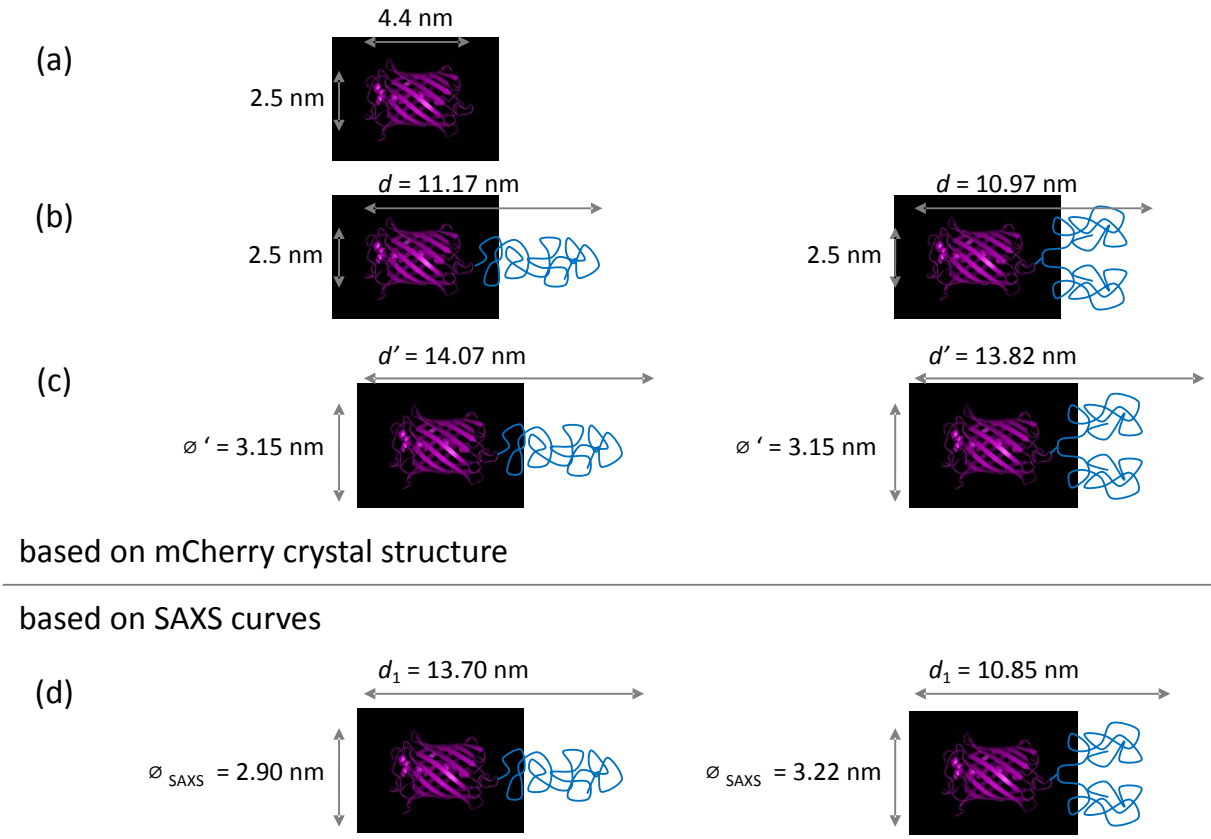


Figure S29. Size of one bioconjugates. (a) mCherry based upon crystal structure, (b) calculated size of bioconjugates based upon mCherry crystal structure, (c) calculated swollen size of bioconjugates based upon mCherry crystal structure, (d) calculate size of bioconjugates based upon SAXS curves at 50 wt%, 10 °C.

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