

Supplementary Information

Site-dependent fluorescence enhanced polymers with a self-restricted GFP chromophore for living cells imaging

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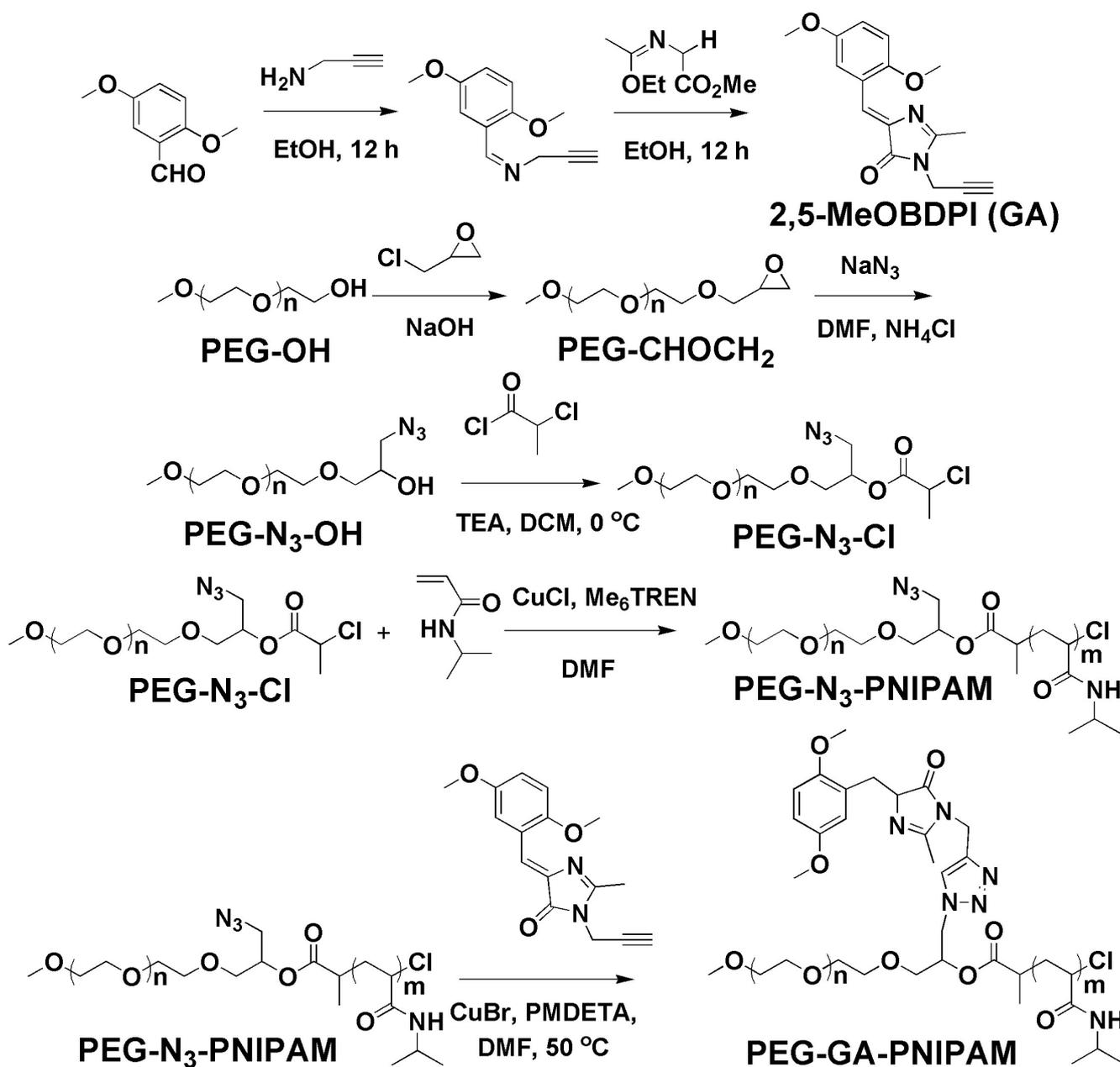
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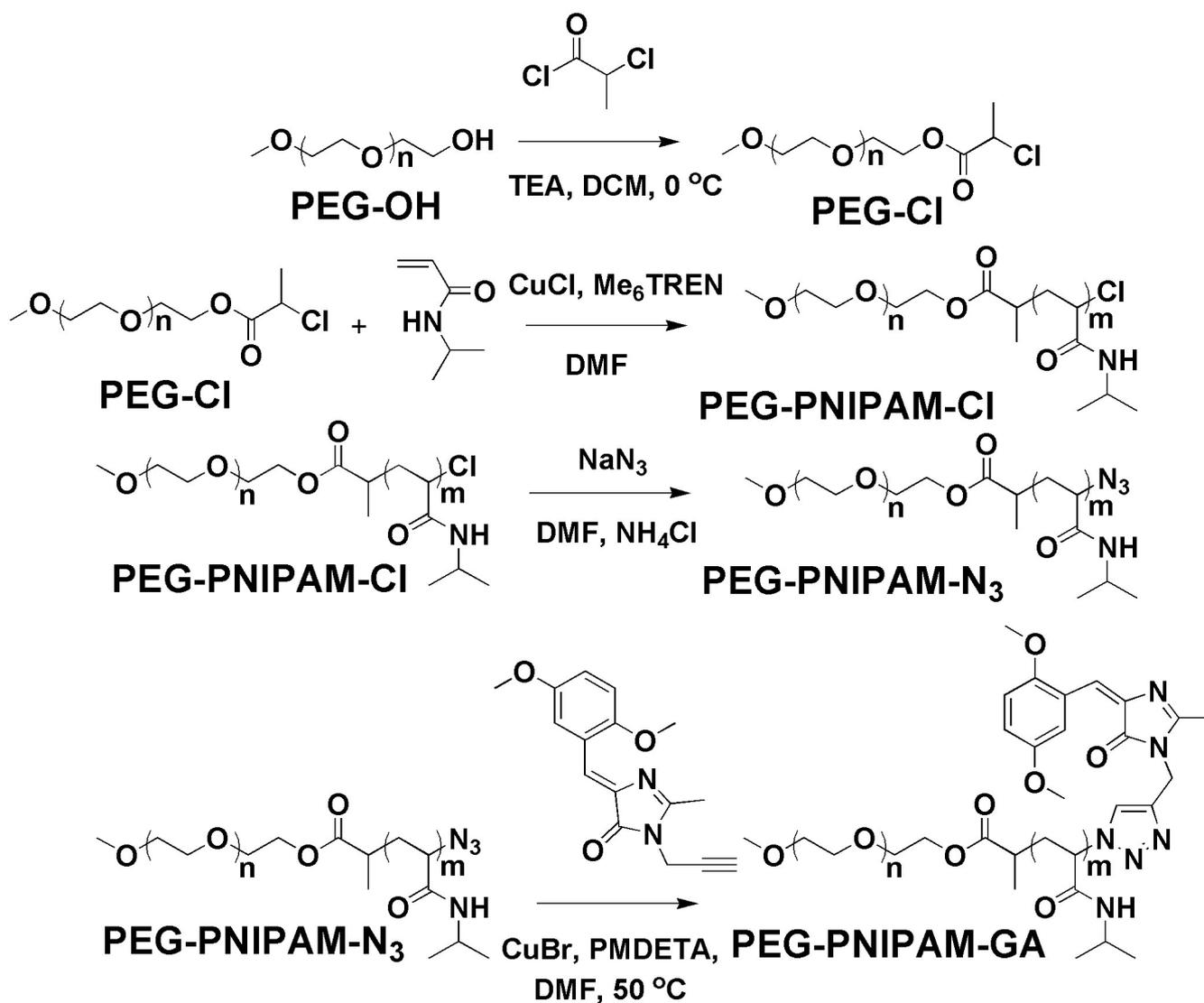
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S-1 Characterization of GFP chromophore GA, PEG-Cl, PEG-CHOCH₂, PEG-N₃-OH, PEG-N₃-Cl, PEG-N₃-PNIPAM, PEG-GA-PNIPAM, PEG-Cl, PEG-PNIPAM-Cl, PEG-PNIPAM-N₃, PEG-PNIPAM-GA

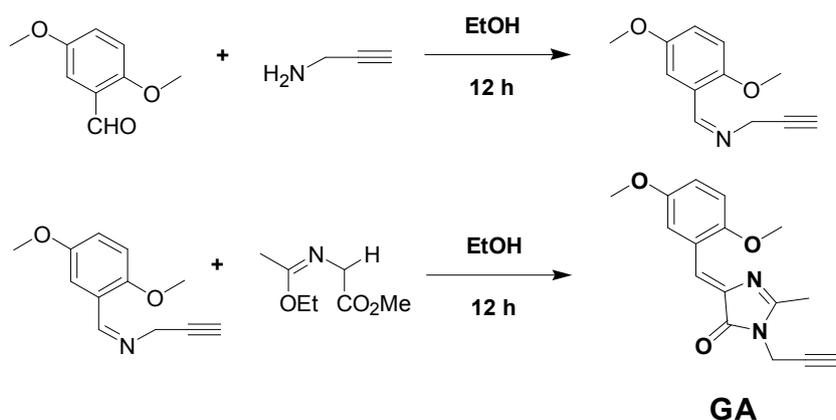


Scheme S1. The synthesis route of PEG-GA-PNIPAM.



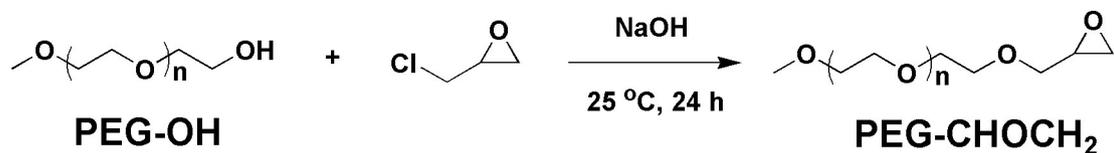
Scheme S2. S The synthesis route of PEG-PNIPAM-GA.

Synthesis of Chromophore GA



The chromophore GA was synthesized as reported.¹ Schiff bases were prepared by combining 2,5-dimethoxybenzal (166 mg, 1 mmol) with propargylamine (55 mg, 1.1 mmol) in ethanol for 12 h at room temperature. Then, the solvent was condensed under vacuum and methyl 2-(1-ethoxyethylideneamino) acetate (175 mg, 1.1 mmol) was added in ethanol. The reaction was allowed to stir overnight under ambient conditions. The progression of reactions was monitored by thin-layer-chromatography. Finally, the mixture was purified by silica gel column chromatography using ethyl acetate and petroleum ether as the solvents. Yield = 61%. ¹H NMR (400 MHz, CDCl₃, 298 K): δ (ppm): 8.44 (d, J = 3.2 Hz, 1H), 7.70 (s, 1H), 6.95 (dd, J = 9.2 Hz, J = 3.2 Hz, 1H), 6.85 (d, J = 9.2 Hz, 1H), 4.43 (d, J = 2.4 Hz, 2H), 3.87 (s, 3H), 3.86 (s, 3H), 2.52 (s, 3H), 2.31 (t, J = 2.6 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃, ppm, 298 K): 169.54, 160.94, 154.19, 153.67, 137.86, 123.75, 122.24, 118.49, 117.18, 112.08, 77.56, 72.75, 56.42, 56.00, 29.57, 16.11. IR (KBr, cm⁻¹): 3250, 3221, 3083, 2989, 2953, 2933, 2905, 2825, 2116, 1712, 1644, 1578, 1568, 1493, 1460, 1437, 1426, 1418, 1401, 1367, 1341, 1320, 1304, 1285, 1260, 1241, 1222, 1194, 1172, 1145, 1085, 1058, 1023, 942, 929, 920, 907, 889, 808, 800, 769, 739, 709, 685, 664, 614, 563, 546, 533, 519, 491.

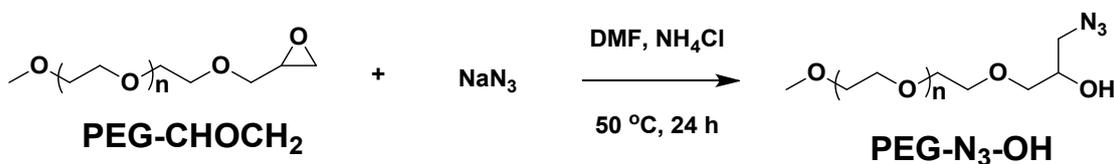
Synthesis of PEG-CHOCH₂



PEG-OH ($M_n=2000$ g/mol, 10 g, 5 mmol) and NaOH (20 g, 500 mg) were dissolved in 60 mL 2-(chloromethyl)oxirane. Subsequently, the mixture was stirred at 25 °C for 24 h.

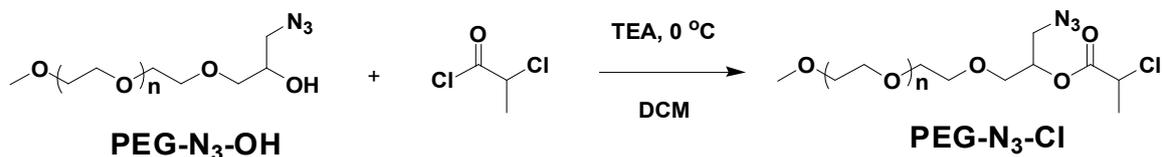
The mixture was filtered. Then, it was precipitated into excess of cold diethyl ether, filtered and redissolved in CH₂Cl₂. It was precipitated in cold diethyl ether for three times. Finally, the product was concentrated in a vacuum oven at 40 °C for 24 h to obtain a white powdered solid. Yield = 30%. ¹H NMR (400 MHz, CDCl₃, 298 K): δ (ppm): 3.39-3.82 (br, -OCH₂CH₂O-), 3.37 (s, CH₃O-), 3.18-3.14 (br, CH₂OCH-), 2.80-2.78 (br, -OCH₂-), 2.61-2.59 (br, -OCH₂-). IR (KBr, cm⁻¹): 3435, 2890, 2318, 1967, 1741, 1636, 1467, 1362, 1344, 1281, 1243, 1147, 1116, 1061, 964, 948, 843, 720, 532.

Synthesis of PEG-N₃-OH



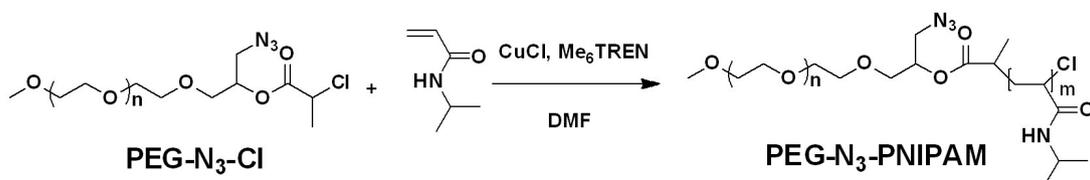
PEG-CHOCH₂ (5 g, 2.5 mmol), NaN₃ (1.30 g, 20 mmol) and NH₄Cl (1.07 g, 20 mmol) were dissolved in 40 mL DMF under nitrogen protection. The mixture was stirred at 50 °C for 24 h. Condense the DMF by reduced pressure distillation, and add CH₂Cl₂ (5 mL) to the mixture. Then it was precipitated into excess of cold diethyl ether and collected by filtration. It was precipitated in cold diethyl ether for three times. Finally, the product was concentrated in a vacuum oven at 40 °C for 24 h to obtain a white powdered solid. Yield = 61%. ¹H NMR (400MHz, DMSO-*d*₆, 298 K): δ (ppm): 5.25-5.23 (d, -OH), 3.78-3.72 (br, N₃CH₂-), 3.68-3.63 (br, N₃CH₂CH(OH)CH₂-), 3.57-3.30 (br, -OCH₂CH₂O-), 3.22 (s, CH₃O-). IR (KBr, cm⁻¹): 3435, 2889, 2101, 1963, 1634, 1468, 1360, 1344, 1281, 1243, 1149, 1115, 1061, 964, 843, 529.

Synthesis of PEG-N₃-Cl



PEG-N₃-OH (2 g, 1 mmol), triethylamine (0.7 mL, 5 mmol) and triethylamine (0.36 mL, 2.6 mmol) were dissolved in 30 mL CHCl₂ under nitrogen protection and cooled to 0 °C. 2-Chloropropionyl chloride (0.48 mL, 5 mmol) and CH₂Cl₂ (10 mL) were added dropwise, respectively. The mixture was kept for 24 h and diluted with CH₂Cl₂ (30 mL), washed with DI water (5 mL) and concentrated under reduced pressure. Then it was precipitated into cold diethyl ether three times to obtain a white solid. Yield = 82%. ¹H NMR (400 MHz, DMSO-*d*₆, 298 K): δ (ppm): 5.11-5.04 (br, CH₃CHCl-), 4.77-4.69 (br, -OCH₂CH(N₃)O-), 3.66-3.28 (br, -OCH₂CH₂O-), 3.21 (s, CH₃O-), 1.67-1.53 (br, CH₃-), 1.21-1.11 (br, N₃CH₂-). IR (KBr, cm⁻¹): 3435, 2887, 2104, 1745, 1636, 1467, 1360, 1344, 1280, 1243, 1145, 1116, 1062, 964, 948, 843, 527.

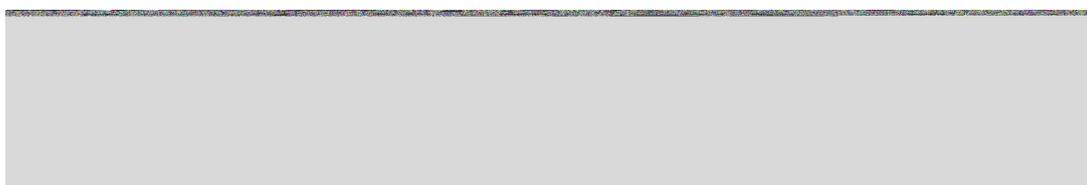
Synthesis of PEG-N₃-PNIPAM₅₉



PEG-c-PNIPAM was synthesized by atom transfer radical polymerization (ATRP) using PEG-c-Cl as the macroinitiator. PEG-N₃-Cl (0.4 g, 0.2 mmol) and NIPAM (1.808 g, 16 mmol) were added into a reaction flask under nitrogen protection, and then 4 mL of DMF was added and degassed under nitrogen purge. Subsequently, Me₆TREN (54 μL,

0.2 mmol) was added and degassed under nitrogen purge. At last, CuCl (20 mg, 0.2 mmol) was added into the flask and degassed under nitrogen purge, polymerization was performed at 30 °C for 10 h. Condense the DMF by reduced pressure distillation. The product was solubilized in THF and purified through an aluminum column to remove the copper complex. After the solution was concentrated, it was precipitated into cold diethyl ether for three times. Finally, it was filtered and dried at 40 °C for 24 h to obtain a white solid. Yield = 54%. ¹H NMR (400 MHz, CDCl₃, 298 K): δ (ppm): 6.72-6.07 (br, -CHNHCO-), 3.99 (br, -CHNHCO-), 3.74-3.43 (br, -OCH₂CH₂O-), 3.37 (s, CH₃O-), 2.71 (br, -CH₂CHCONH-), 2.38-1.51 (br, -CH₂CHCONH-), 1.13 (br, -CH(CH₃)₂). ¹³C NMR (100 MHz, CDCl₃, 298K): δ (ppm): 174.8, 77.6, 77.3, 77.0, 72.1, 70.7, 59.2, 42.6, 41.5, 35.5, 29.9, 22.8. IR (KBr, cm⁻¹): 3436, 3309, 3070, 2972, 2930, 2875, 2104, 1648, 1545, 1459, 1386, 1367, 1252, 1172, 1131, 1110, 953, 881, 840, 668. GPC: Mn=8.9×10³ g/mol, PDI = 1.24.

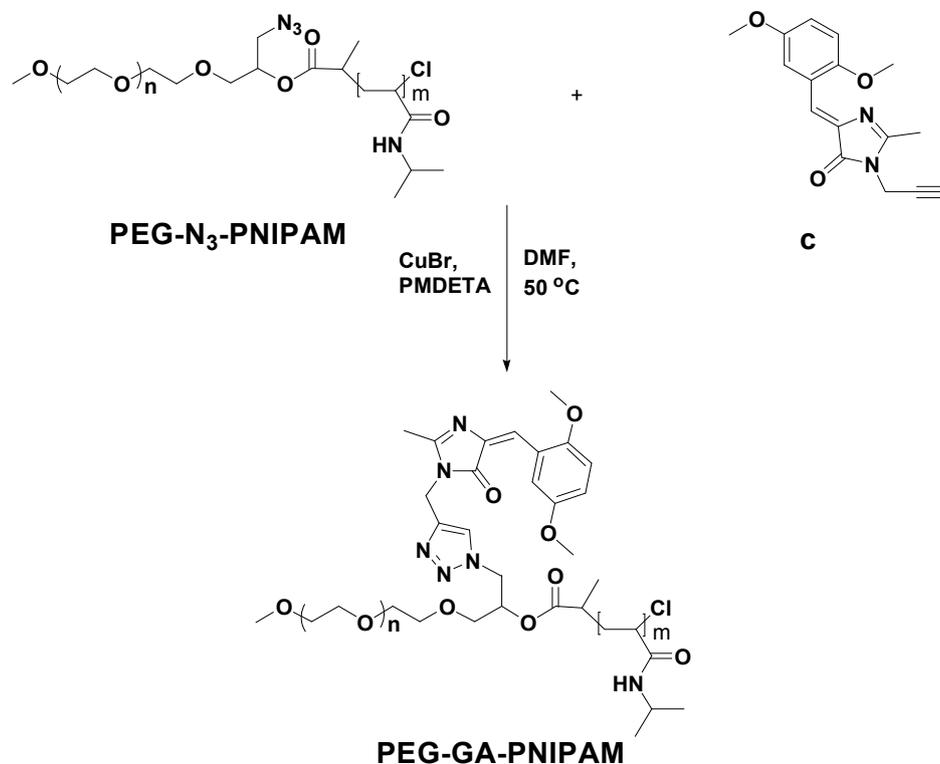
Synthesis of PEG-N₃-PNIPAM₁₀₆



PEG-c-PNIPAM was synthesized by atom transfer radical polymerization (ATRP) using PEG-c-Cl as the macroinitiator. PEG-N₃-Cl (0.3 g, 0.15 mmol) and NIPAM (2.547 g, 22.5 mmol) were added into a reaction flask under nitrogen protection, and then 4.5 mL of DMF was added and degassed under nitrogen purge. Subsequently, Me₆TREN (41 μL,

0.15 mmol) was added and degassed under nitrogen purge. At last, CuCl (15 mg, 0.15 mmol) was added into the flask and degassed under nitrogen purge, polymerization was performed at 30 °C for 10 h. Condense the DMF by reduced pressure distillation. The product was solubilized in THF and purified through an aluminum column to remove the copper complex. After the solution was concentrated, it was precipitated into cold diethyl ether for three times. Finally, it was filtered and dried at 40 °C for 24 h to obtain a white solid. Yield = 56%. ¹H NMR (400 MHz, CDCl₃, 298 K): δ (ppm): 6.96-6.01 (br, -CHNHCO-), 3.99 (br, -CHNHCO-), 3.78-3.42 (br, -OCH₂CH₂O-), 3.37 (s, CH₃O-), 2.45-1.99 (br, -CH₂CHCONH-), 1.98-1.51 (br, -CH₂CHCONH-), 1.14 (br, -CH(CH₃)₂). ¹³C NMR (100 MHz, CDCl₃, 298K): δ (ppm): 174.7, 77.6, 77.2, 76.9, 72.1, 70.7, 59.3, 42.5, 41.5, 35.5, 22.8. IR (KBr, cm⁻¹): 3437, 3312, 3073, 2973, 2932, 2876, 2105, 1651, 1547, 1460, 1387, 1368, 1252, 1173, 1131, 928, 882, 840, 663. GPC: Mn=1.42×10⁴ g/mol, PDI=1.22.

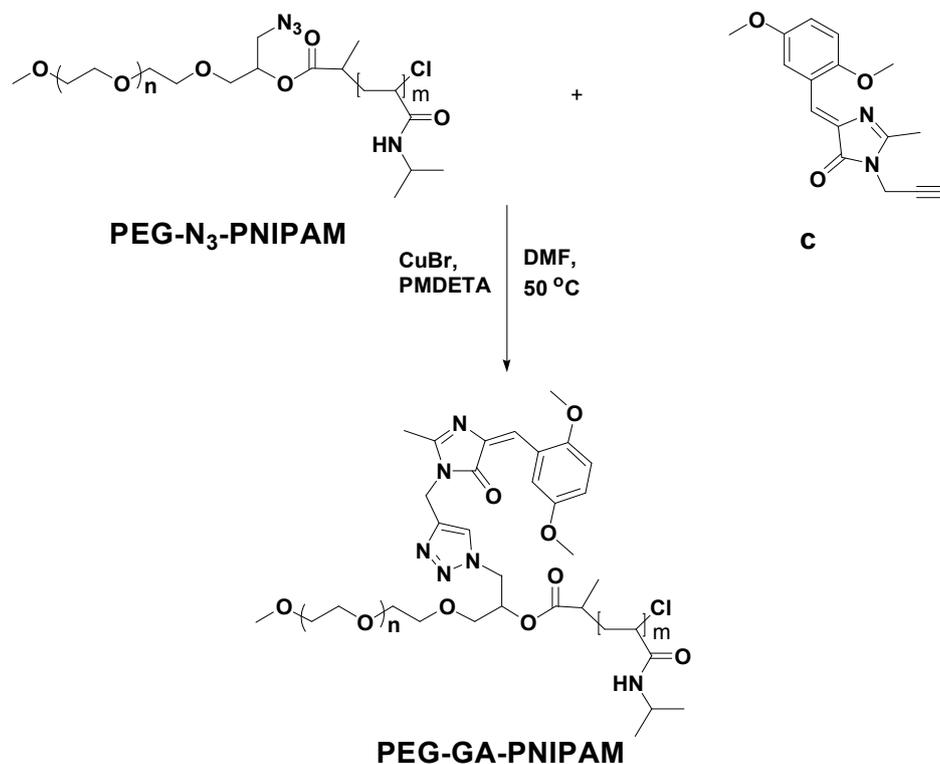
Synthesis of PEG-GA-PNIPAM₅₉



PEG-N₃-PNIPAM₅₉ (400 mg, 0.05 mol), GA (16 mg, 0.055 mmol), PMDETA (12 μ L, 0.055 mmol) were dissolved in 5 mL DMF under nitrogen protection in a reaction flask. CuBr (9 mg, 0.063 mmol) was added and degassed, reaction was performed at 50 °C for 24 h. to remove the copper complex, the product remained was solubilized in THF and purified through an aluminum column. Concentrate the solution, and precipitate it into -20 °C diethyl ether for three times. Finally, it was filtered and dried at 40 °C for 24 h to obtain a yellow solid. Yield = 70%. ¹H NMR (400 MHz, CDCl₃, 298 K): δ (ppm): 6.82-5.97 (br, -CHNHCO-), 3.99 (br, -CHNHCO-), 3.76-3.43 (br, -OCH₂CH₂O-), 3.37 (s, CH₃O-), 2.71 (br, -CH₂CHCONH-), 2.38-1.51(br, -CH₂CHCONH-), 1.13 (br, -CH(CH₃)₂). IR (KBr, cm⁻¹): 3436, 3305, 3079, 2972, 2925, 2875, 2856, 2106, 1647, 1549, 1460, 1386, 1368, 1244,

1172, 1131, 1111, 953, 841, 694, 519.

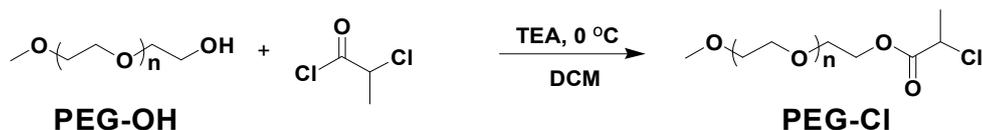
Synthesis of PEG-GA-PNIPAM₁₀₆



PEG-N₃-PNIPAM₁₀₆ (750 mg, 0.05 mol), GA (16 mg, 0.055 mmol), PMDETA (12 μ L, 0.055 mmol) were dissolved in 5 mL DMF under nitrogen protection in a reaction flask. CuBr (9 mg, 0.063 mmol) was added and degassed, and reaction was performed at 50 °C for 24 h. To remove the copper complex, the product remained was solubilized in THF and purified through an aluminum column. Concentrate the solution, and precipitate it into -20 °C diethyl ether for three times. Finally, it was filtered and dried at 40 °C for 24 h to obtain a yellow solid. Yield = 70%. ¹H NMR (400 MHz, CDCl₃, 298 K): δ (ppm): 6.96-6.01 (br, -CHNHCO-), 3.99 (br, -CHNHCO-), 3.78-3.42 (br, -OCH₂CH₂O-), 3.38 (s, CH₃O-), 2.45-1.99 (br, -CH₂CHCONH-), 1.98-1.51 (br, -CH₂CHCONH-), 1.14 (br, -CH(CH₃)₂). IR (KBr,

cm⁻¹): 3437, 3308, 3080, 2974, 2926, 2876, 2855, 2107, 1647, 1551, 1461, 1386, 1368, 1256, 1172, 1131, 1112, 930, 840, 711, 514.

Synthesis of PEG-Cl



PEG-OH (2 g, 1 mmol) and triethylamine (0.7 mL, 5 mmol) were dissolved in 30 mL CHCl₂ under nitrogen protection and cooled to 0 °C. Triethylamine (0.36 mL, 2.6 mmol) and 10 mL CH₂Cl₂ were added dropwise, respectively. The mixture was kept for 24 h and diluted with CH₂Cl₂ (30 ml), washed with DI water (5 mL). Then it was concentrated under reduced pressure, and precipitated into cold diethyl ether for three times. Finally, it was filtered and dried at 40 °C to obtain a white solid. Yield = 80%. ¹H NMR (400 MHz, CDCl₃, 298 K): δ (ppm): 5.19-5.09 (br, CH₃CHCl-), 4.49-4.39 (br, -CH₂OCO-), 4.00-3.40 (br, -OCH₂CH₂O-), 3.38 (s, CH₃O-), 1.71-1.63 (br, CH₃-). IR (KBr, cm⁻¹): 3434, 2889, 1967, 1747, 1636, 1468, 1455, 1360, 1344, 1281, 1243, 1149, 1114, 1061, 964, 947, 843.

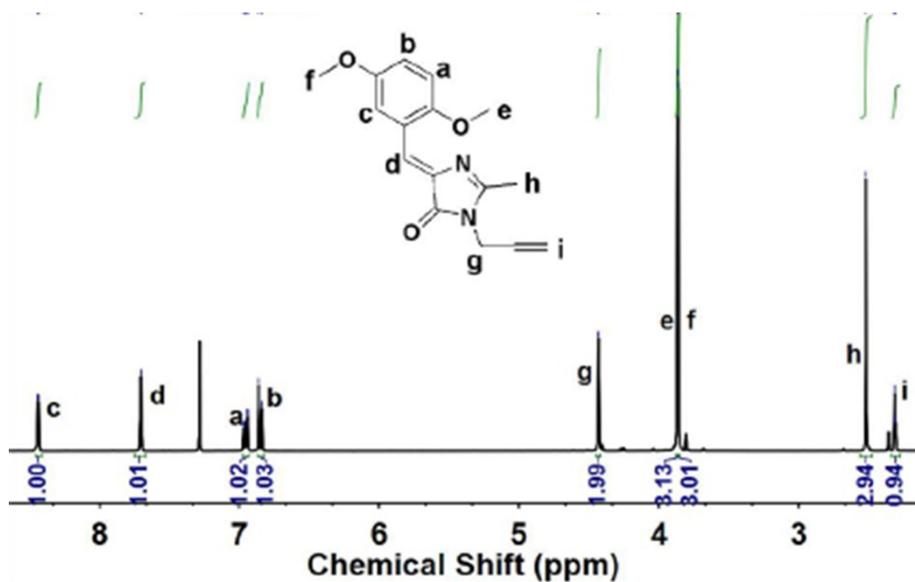


Figure S1. ¹H NMR spectrum of the chromophore in CDCl₃.

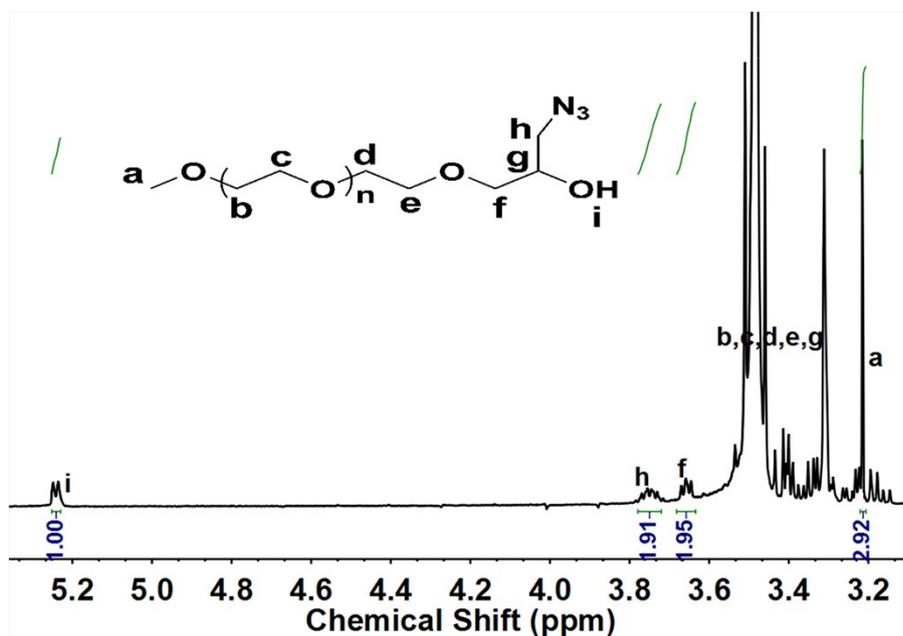


Figure S2. ¹H NMR spectrum of PEG-CHOCH₂ in CDCl₃.

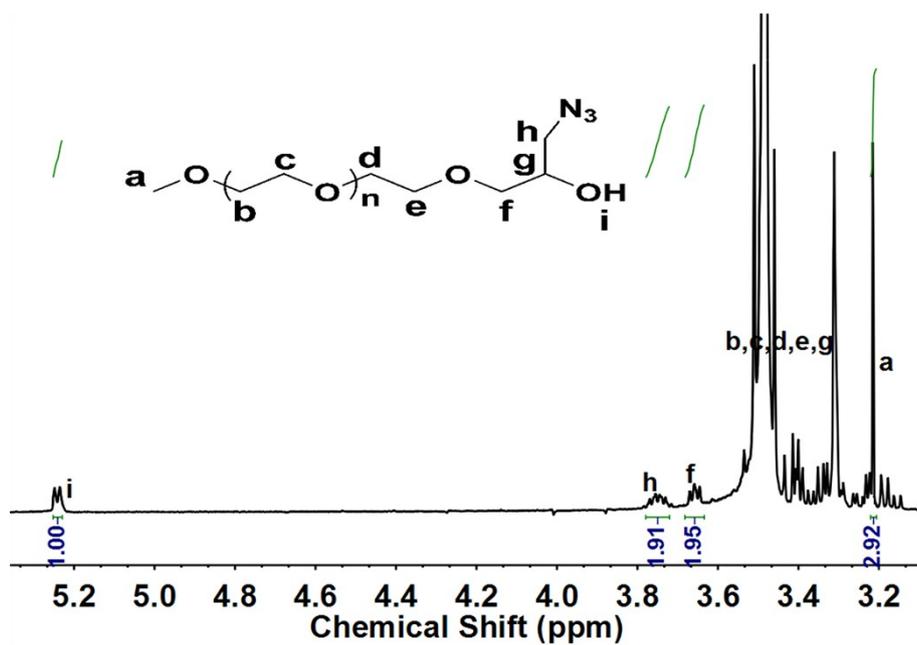


Figure S3. ¹H NMR spectrum of PEG-N₃-OH in DMSO-d₆.

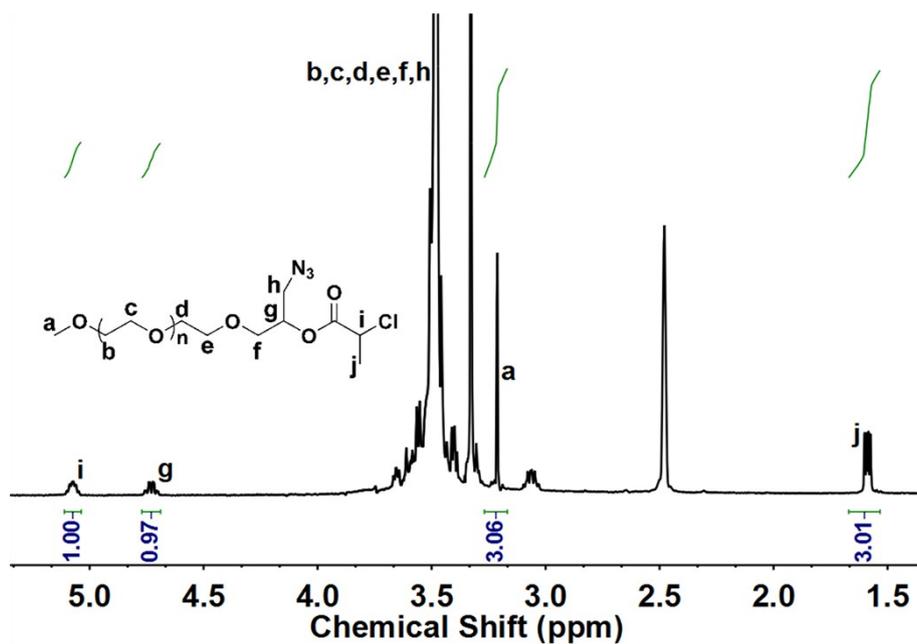


Figure S4. ¹H NMR spectrum of PEG-N₃-Cl in DMSO-d₆.

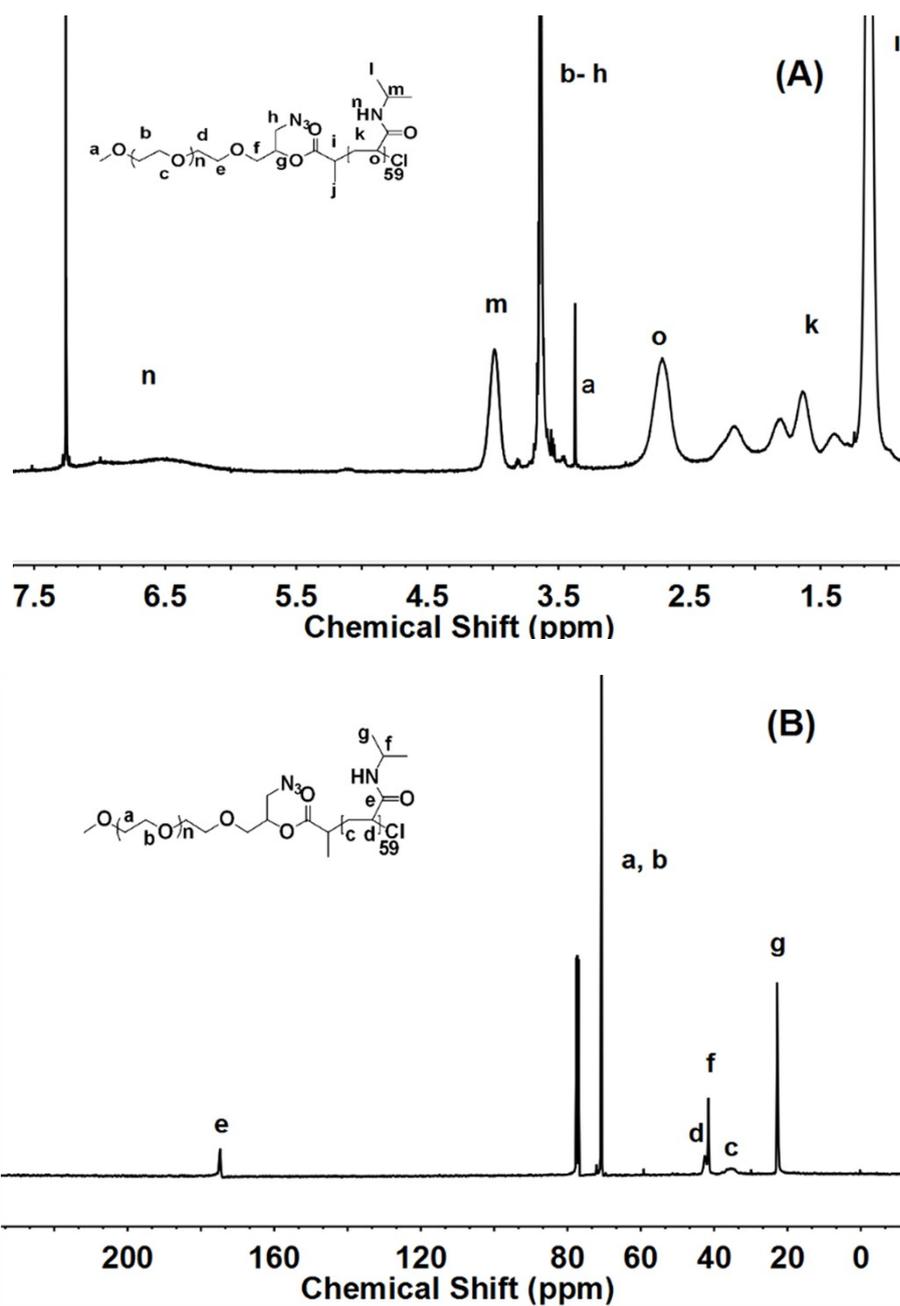


Figure S5. ^1H NMR (A) and ^{13}C NMR (B) spectrum of PEG- N_3 -PNIPAM $_{59}$ in CDCl_3 .

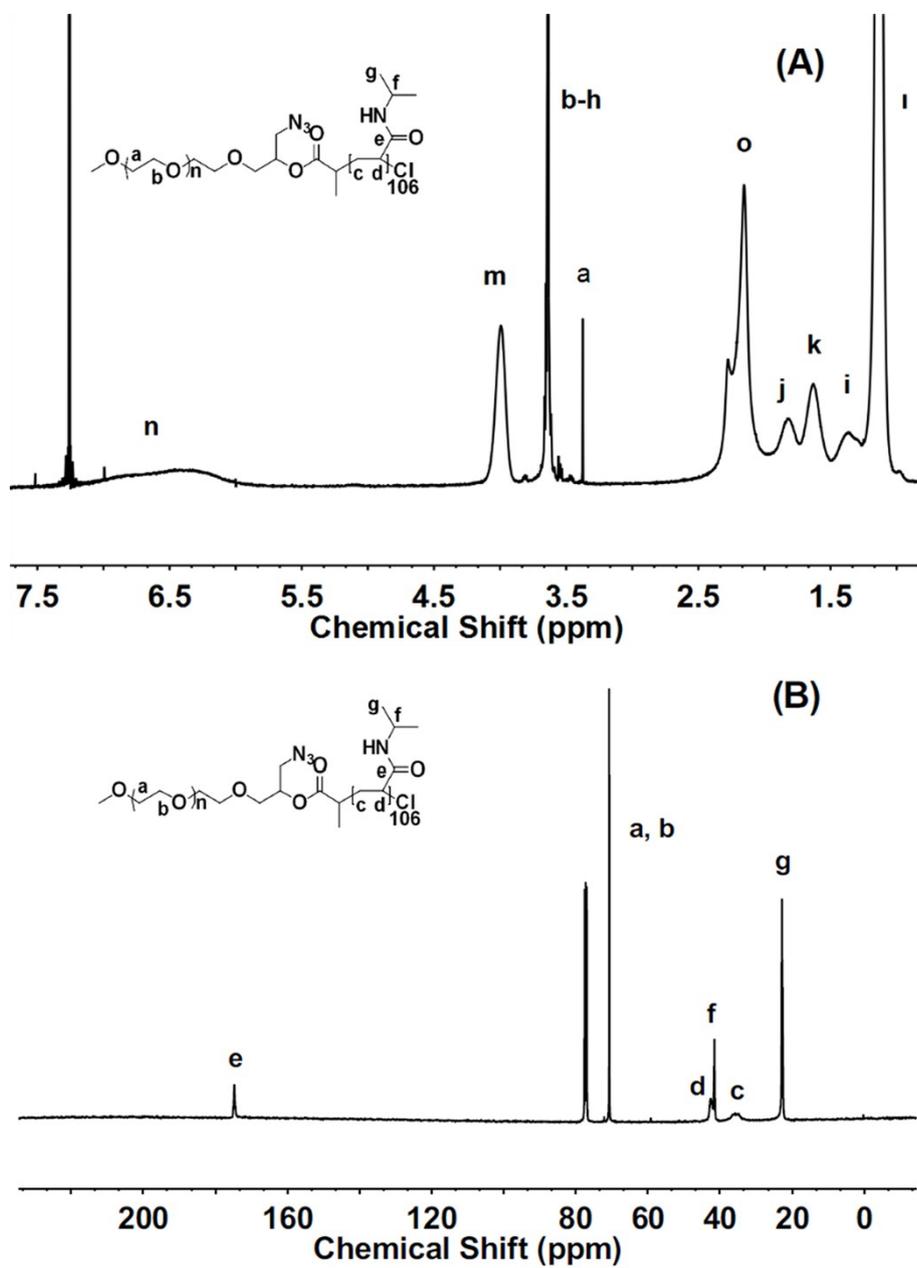


Figure S6. ^1H NMR (A) and ^{13}C NMR (B) spectrum of PEG- N_3 -PNIPAM $_{106}$ in CDCl_3 .

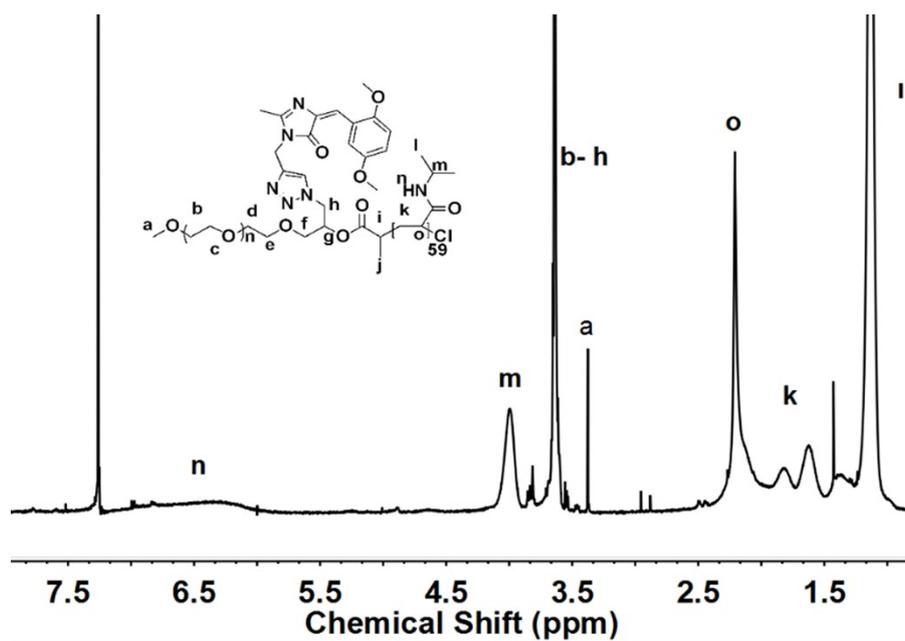


Figure S7. ¹H NMR spectrum of PEG-GA-PNIPAM₅₉ in CDCl₃.

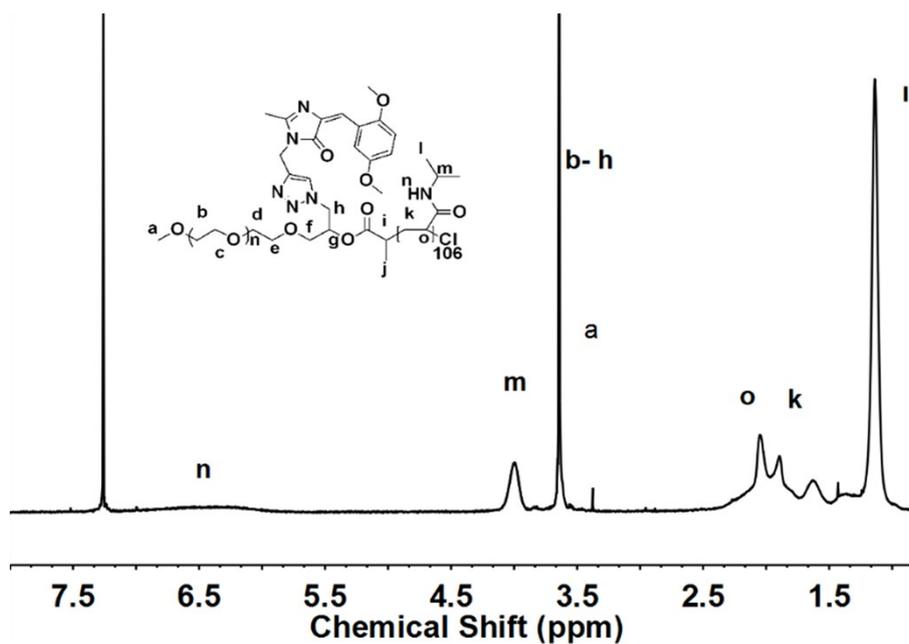


Figure S8. ¹H NMR spectrum of PEG-GA-PNIPAM₁₀₆ in CDCl₃.

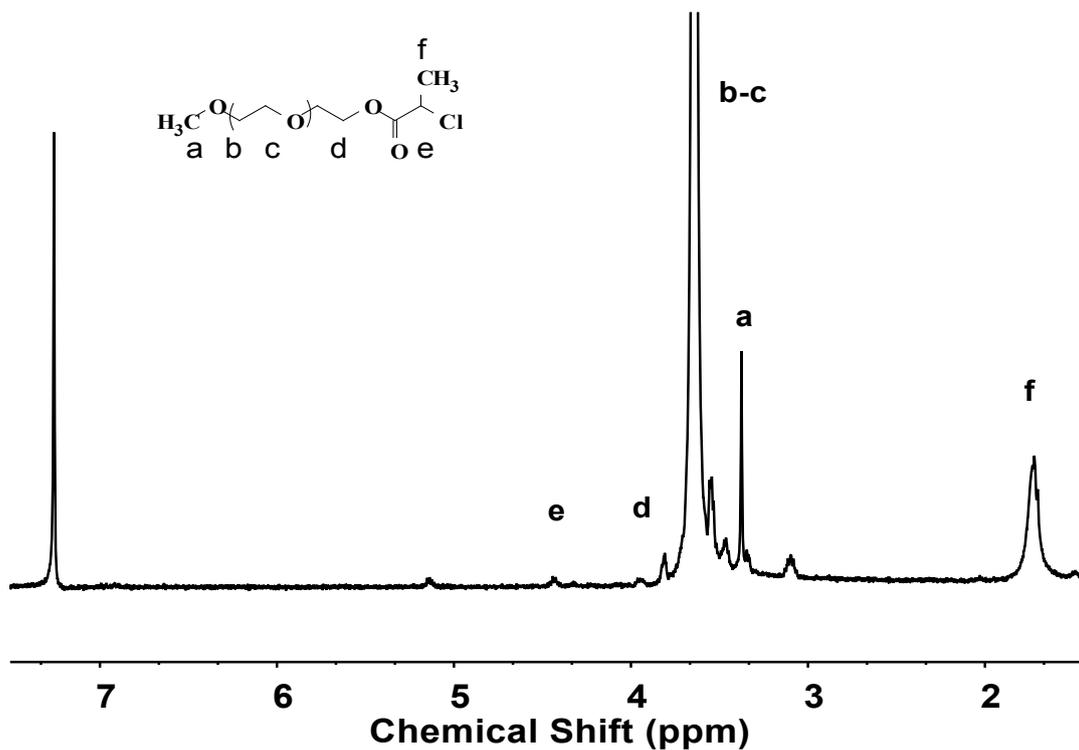


Figure S9. ^1H NMR spectrum of PEG-Cl in CDCl_3 .

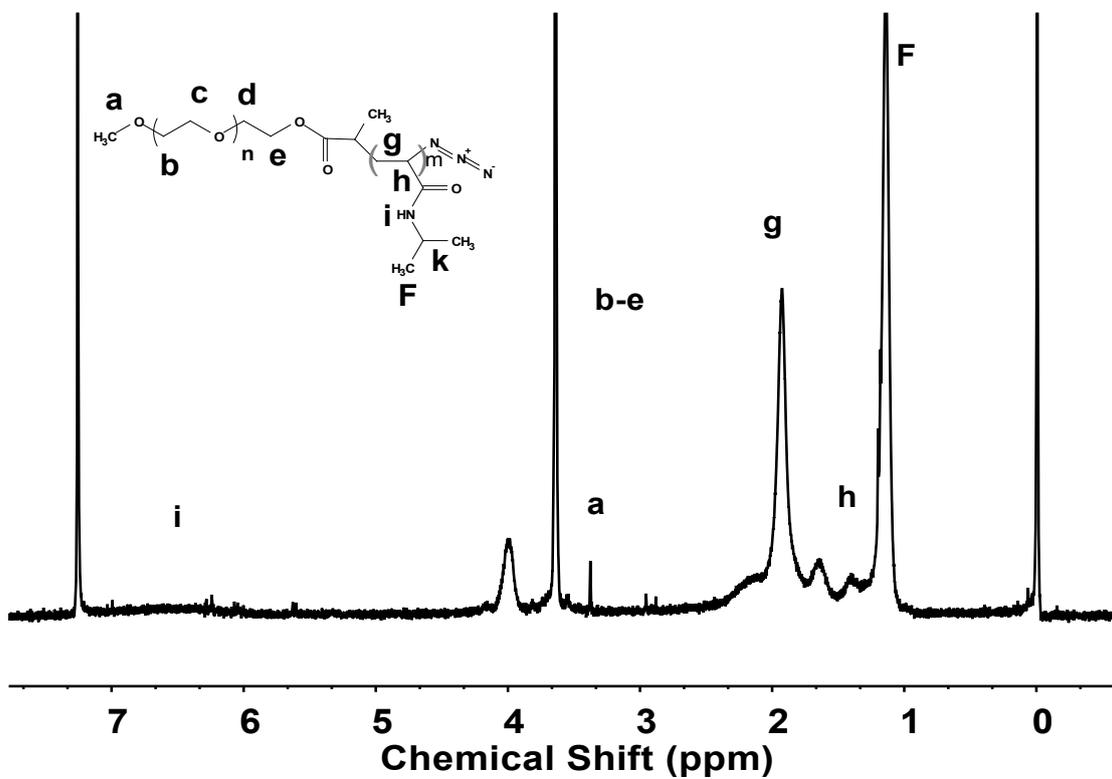


Figure S10. ^1H NMR spectrum of PEG-PNIPAM- N_3 in CDCl_3 .

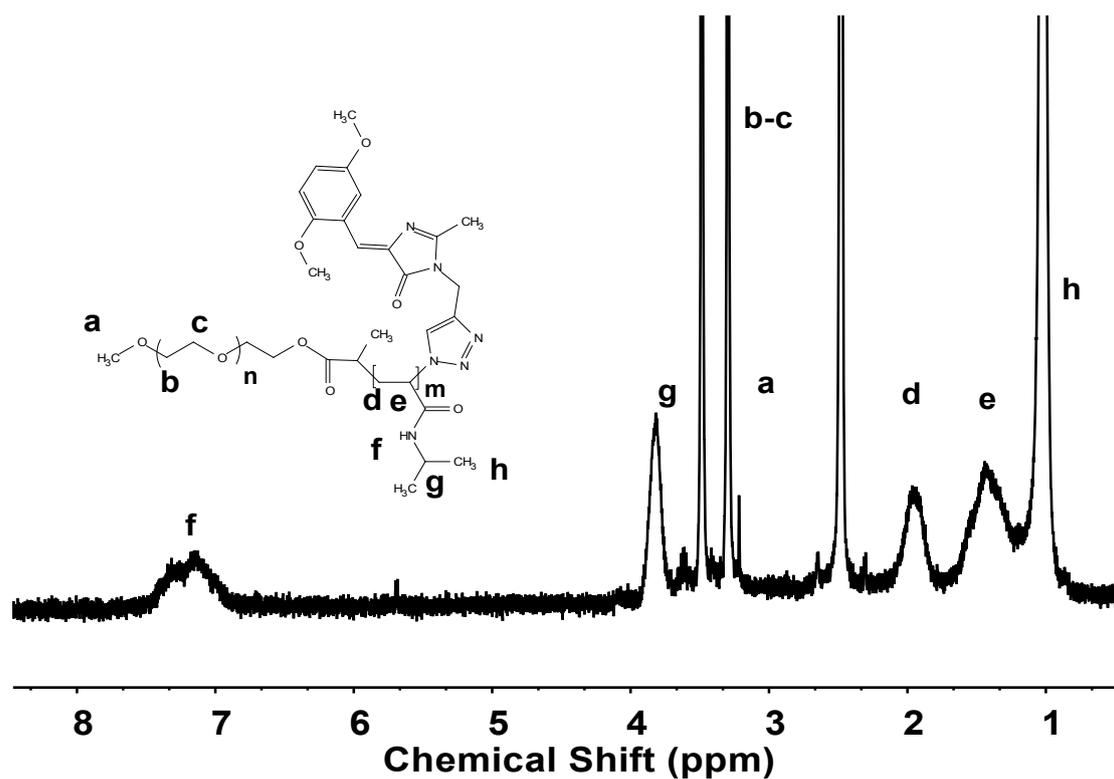


Figure S11. ^1H NMR spectrum of PEG-PNIPAM-GA in CDCl_3 .

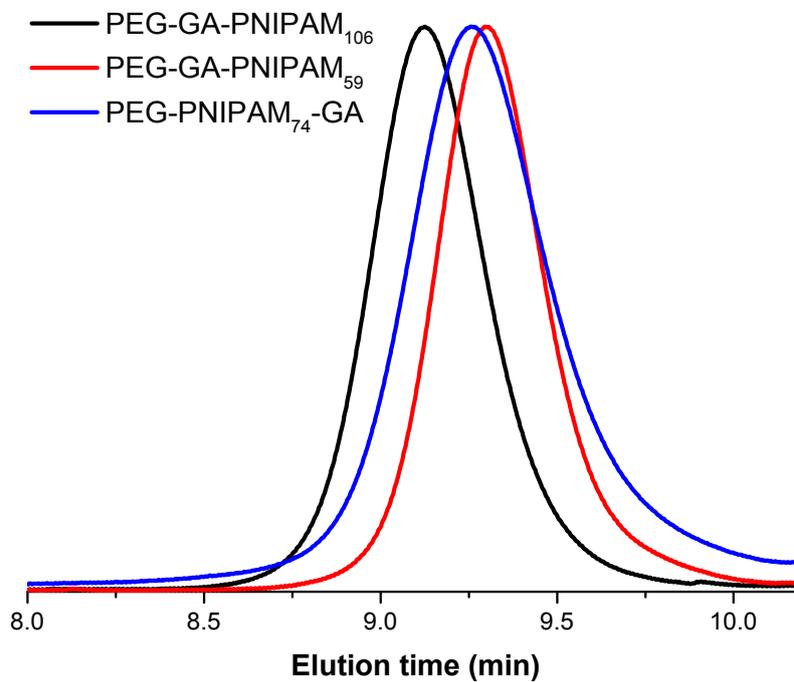


Figure S12. Gel permeation chromatography (GPC) curves of PEG-GA-PNIPAM₁₀₆, PEG-GA-PNIPAM₅₉ and PEG-PNIPAM₇₄-GA.

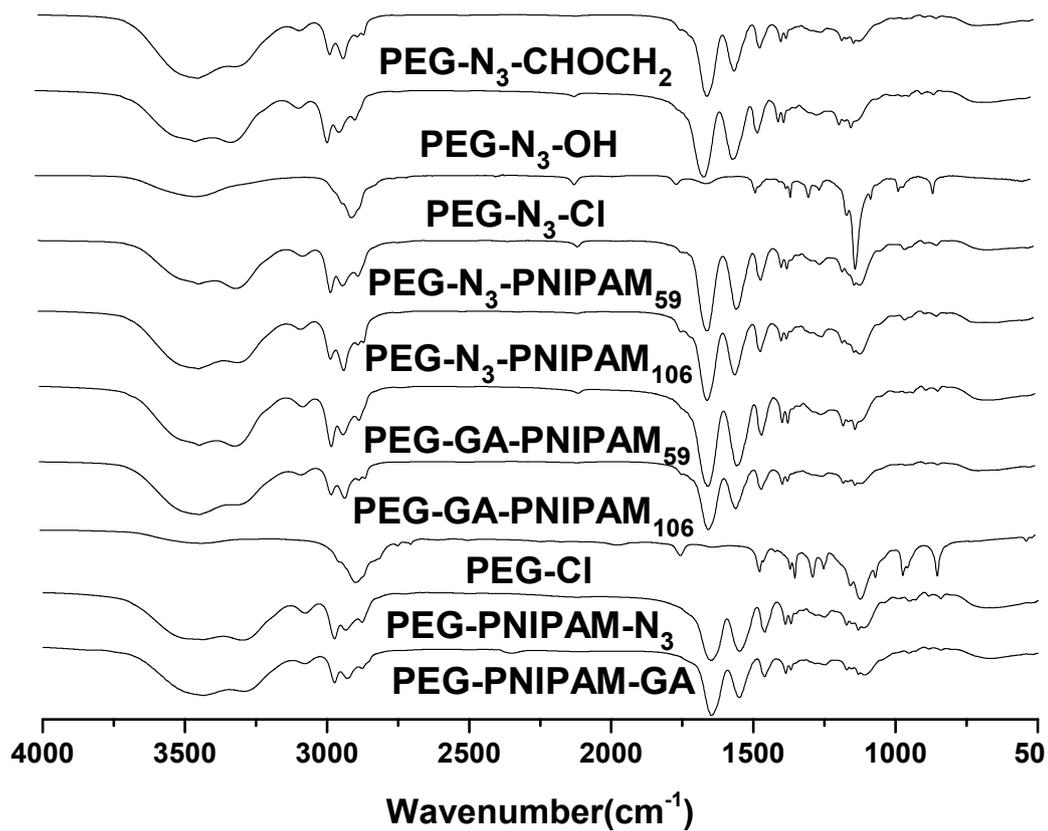


Figure S13. FT-IR spectrum of PEG-CHOCH₂, PEG-N₃-Cl, PEG-N₃-OH, PEG-N₃-PINPAM₅₉, PEG-GA-PINPAM₅₉, PEG-N₃-PINPAM₁₀₆, PEG-GA-PINPAM₁₀₆, PEG-Cl, PEG-PMIPAM-N₃, PEG-PNIPAM-GA.

S-2 Fluorescence of PEG-PNIPAM₇₄-GA

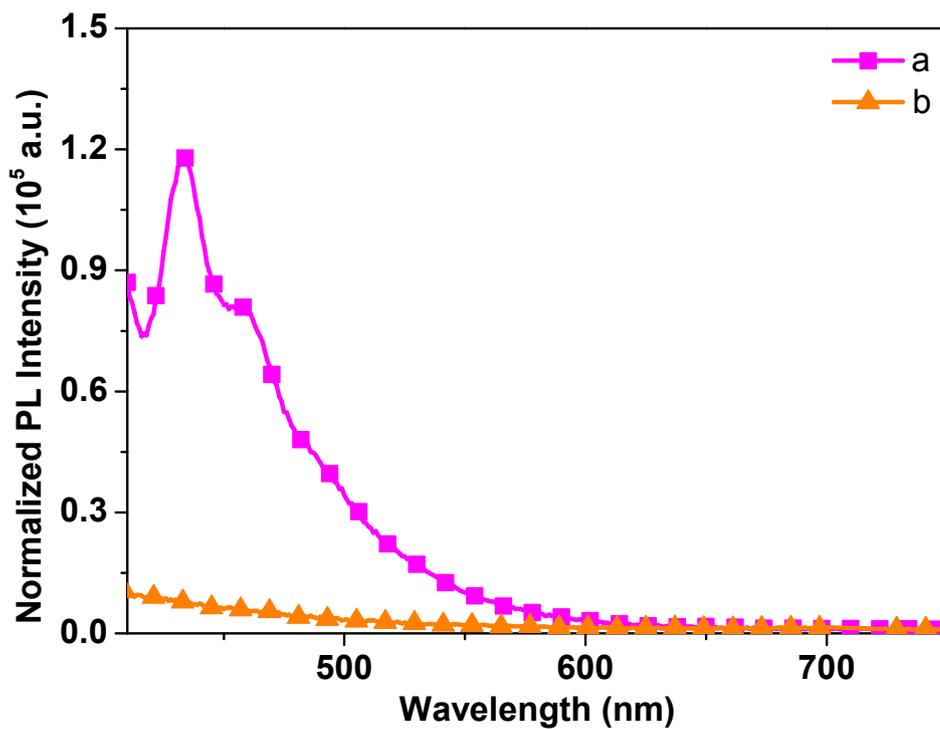


Figure S14. PL intensity of PEG-PNIPAM₇₄-GA in THF (a) and H₂O (b). $\lambda_{\text{ex}} = 397$ nm

Concentration was 1mg/mL.

S-3 Fluorescence of PEG-GA-PNIPAM₅₉ and PEG-GA-PNIPAM₁₀₆ in the cooling and heating cycles

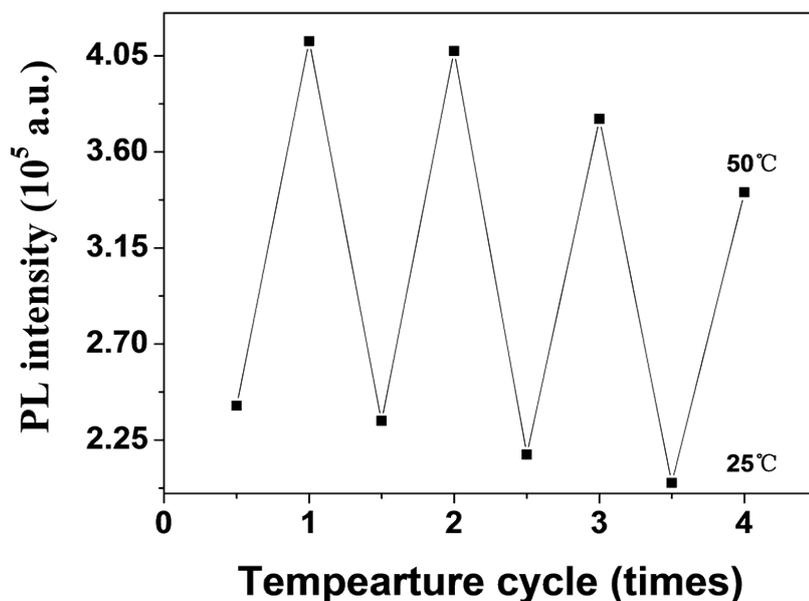


Figure S15. PL intensity of PEG-GA-PNIPAM₅₉ at several heating and cooling cycles in the range of 25 °C to 50 °C. Samples are heated to 50 °C, cooled to 20 °C and heated to 25 °C in a circle. And the stabilization time is 5 minutes. All samples' concentration was 1 mg/mL.

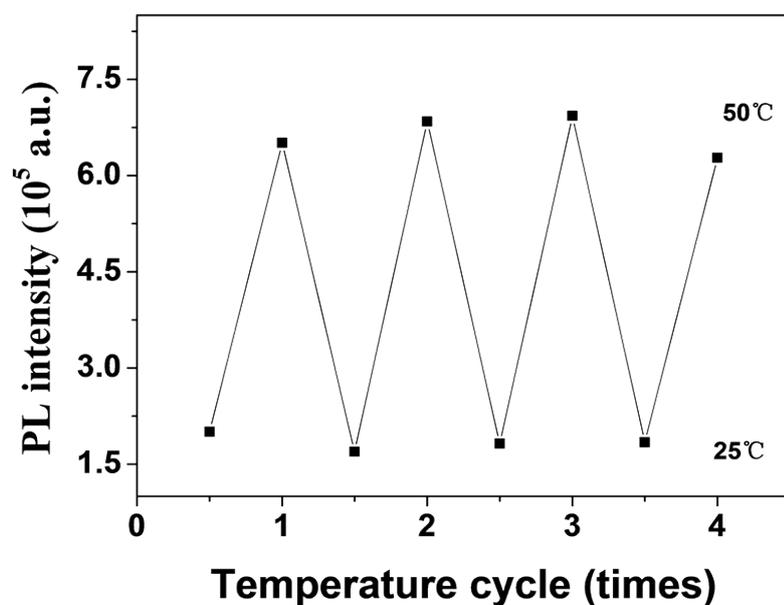


Figure S16. PL intensity of PEG-GA-PNIPAM₁₀₆ at several heating and cooling cycles in the range of 25 °C to 50 °C. Samples are heated to 50 °C, cooled to 20 °C and heated to 25 °C in a circle. And the stabilization time is 5 minutes. All samples' concentration was 1 mg/mL.

S-4 Cytotoxicity of PEG-PNIPAM-GA

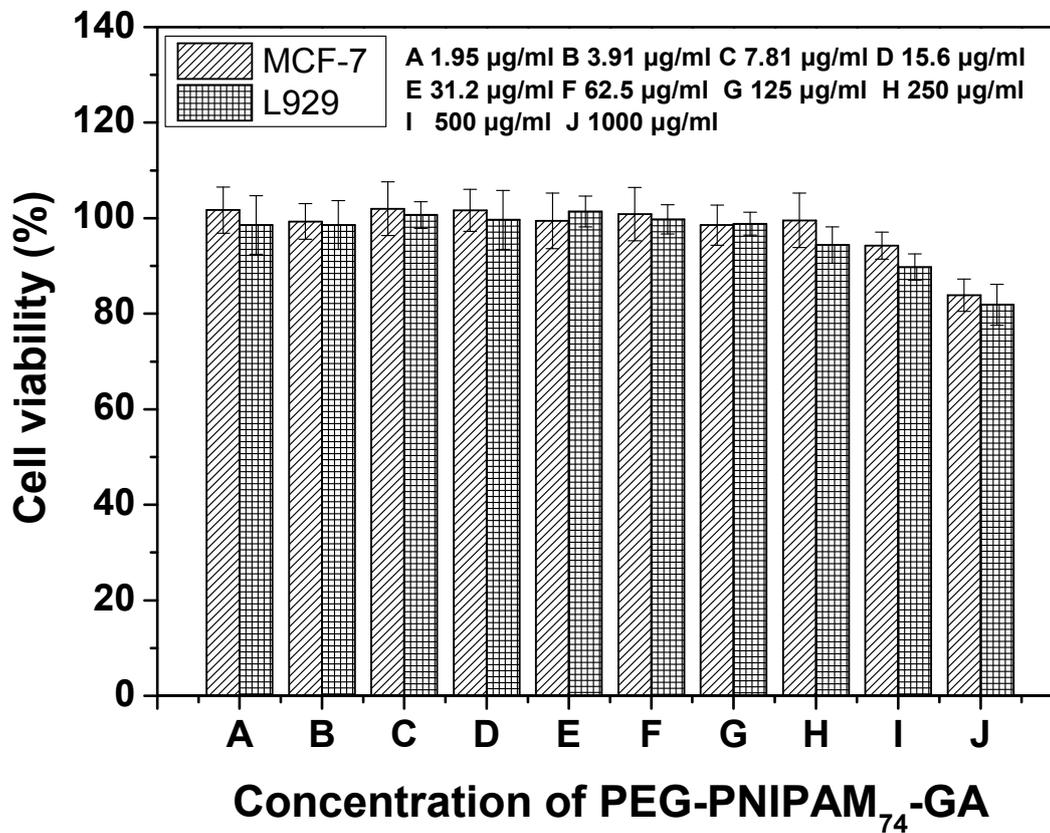


Figure S17. Cell viability of L929 and MCF-7 against PEG-PNIPAM₇₄-GA after cultured for 24 h with different concentration: 1.95 (A), 3.91 (B), 7.81 (C), 15.6 (D), 31.2 (E), 62.5 (F), 125 (G), 250 (H), 500 (I), 1000 mg/mL (J).

References

1. H. Deng, C. Yu, L. Gong, X. Zhu, *J. Phys. Chem. Lett.*, 2016, **7**(15), 2935-2944.