Electronic Supplementary Material (ESI) for RSC Advances.


# SUPPLEMENTARY INFORMATION

## Controlled degradation of low-fouling

## poly(oligo(ethylene glycol) methyl ether methacrylate) hydrogels

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## A. Methods

**PCBMAA copolymer synthesis.** For PCBMAA copolymer synthesis, the CBMAA to AMPA composition was optimized to obtain hydrogels that degrade over 13 and 52 days to achieve short- and long-term degrading hydrogels. PCBMAA-AMPA had a final APMA content of 6 mol%, confirmed by $^1$H NMR (**Fig. S16**). Using a similar procedure to P(EG)$_x$MA derivatization, the amines of PCBMAA-APMA were fully reacted with NHS-AZ derivatives or NHS-DBCO, which was also confirmed by $^1$H NMR (**Figs. S17-S20**) and a fluorescent amine quantification assay.

**CBMAA monomer synthesis.** Carboxybetaine methacrylamide was synthesized according to previously established procedures.[40] Briefly, 7.75 g (46 mmol) of N-[3-(dimethylamino)propyl]-methacrylamide was dissolved in 100 mL of dry acetonitrile under $N_2$. *t*-Butyl bromoacetate (10 g, 51 mmol) was added, and the reaction was kept at 50 °C overnight. The reaction was cooled to room temperature, and the product precipitated in ether (250 mL). The white precipitate was collected by vacuum filtration, washed with ether, and dried overnight in a vacuum oven at 60 °C. The *t*-butyl group was removed by reacting 12 g of the white solid with trifluoroacetic acid (10 mL, 131 mmol) for 2 h at room temperature, followed by precipitation in ether (100 mL). The precipitate was collected, washed with ether, and dried overnight in a vacuum oven at 60 °C. The product was then dissolved in water and lyophilized to yield 8 g (83% yield). $^1$H NMR ($D_2O$, 600 MHz) δ: 5.63 (s, 1H), 5.34 (s, 1H), 4.10 (s, 2H), 3.53 (m, 2H), 3.28 (t, J = 6.42, 2H), 3.18 (s, 6H), 1.96 (m, 2H), 1.85 (s, 3H).

**Synthesis of PCBMAA-APMA (6 mol%)**. Previously synthesized carboxybetaine monomer (2 g, 8.7 mmol) and APMA (40 mg, 0.22 mmol) were dissolved in 1 M acetate buffer pH 5.2. Separately, CTP (5.7 mg, 20 μmol) was dissolved in dioxane and added to the monomer solutions resulting in a 1 M monomer solution of 5:1 acetate buffer:dioxane. pH was adjusted between 3 to 4 using HCl and V-501 (1.15 mg, 4.1 μmol) was added. The reaction mixture was

freeze-pump-thawed (3 times) with a nitrogen backfill and reacted at 70 °C for 24 h. Polymers were purified by dialysis (MWCO 12 -14k) against water at pH ~3 for 3 d and lyophilized to yield a pink powder (2.08 g). Analysis by GPC calibrated with PEG standards determined an $M_n$ of 40.6 kDa and Đ of 1.06. $^1$H NMR analysis indicated an APMA mole fraction of 6 mol% (**Fig. S16**).

**PCBMAA-AZ and PCBMAA-DBCO copolymers**. PCBMAA-APMA was dissolved at 30 mg mL$^{-1}$ in dry methanol and reacted with NHS-AZ derivatives or NHS-DBCO (1.2 eq. relative to amines) and triethylamine (3 eq.) overnight at room temperature. Complete reaction of amines was confirmed by $^1$H NMR (**Fig. S17-S20**). Polymers were purified by dialysis (MWCO 12 -14k) against water at pH ~3 and lyophilized to yield white powders.

**6-Azidohexanal**. A solution of 6-chloro-1-hexanol (14.4 g, 105 mmol) and sodium azide (18.7 g, 288 mmol) in water (200 mL) was refluxed for 20 h. After cooling to room temperature, the mixture was extracted 3 times with ethyl acetate. The combined extracts were washed with brine, dried over $MgSO_4$, filtered, and concentrated to yield 14.7 g (103 mmol, 98%) of 6-azidohexanol, which was used without further purification. Trichloroisocyanuric acid (11.44 g, 48.9 mmol) was added in small portions over 15 min to a vigorously stirred mixture of 6-azido-1-hexanol (14.7 g, 103 mmol), TEMPO (0.206 g, 1.32 mmol), and sodium bicarbonate (13.3 g, 158 mmol) in dichloromethane (200 mL) and water (20 mL). The mixture was stirred for an additional 30 min and filtered through Celite. The organic phase was separated and washed with saturated aqueous $NaHCO_3$ and brine, then dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by flash column chromatography in dichloromethane with a 0 – 5% methanol gradient to yield 6-azidohexanal (8.37 g, 59.3 mmol, 58 %). $^1$H NMR (600 MHz, $CDCl_3$): δ 9.45 (1H, t, J = 1.8 Hz), 3.01 (2H, t, J = 6.9 Hz), 2.17 (2H, dt, J = 1.1, 7.2 Hz), 1.4 – 1.3 (4H, m), 1.2 – 1.1 (2H, m).

**1-(4-Chlorophenylsulfonyl)-7-azido-2-heptanol**. 1.6-M n-butyllithium in hexane (~3 mL, ~4.8

mmol) was added dropwise to a stirred solution of 4-chlorophenyl methyl sulfone (952.2 mg, 4.99 mmol) in anhydrous THF (15 mL) at -78 °C. The mixture was the slowly warmed to 0 °C in an ice bath over ~30 min. The mixture was re-cooled to -78 °C, and 6-azidohexanal (0.78 g, 5.5 mmol) was added to the reaction. After 15 min of stirring, the mixture was left to warm to room temperature. When the mixture became clear, 5 mL of saturated aq. $NH_4Cl$ was added. The mixture was diluted with ethyl acetate and washed successively with water and brine, then dried over $MgSO_4$, filtered, and concentrated to provide the crude product as an oil. Flash column chromatography on silica gel using a gradient of ethyl acetate in hexane (0% - 50 %) yielded a pale-yellow oil (757 mg, 2.29 mmol, 48%). $^1$H-NMR (600 MHz, d6-DMSO): δ 7.90 (2H, d, *J* = 8.4 Hz), 7.70 (2H, d, *J* = 8.4 Hz), 4.83 (1H, d, *J* = 6.1 Hz), 3.86 (1H, m), 3.39 (2H, m), 3.30 (2H, t, d, *J* = 7.2 Hz), 1.5 ~ 1.2 (8H, m).

**1-(4-Methylphenylsulfonyl)-7-azido-2-heptanol.** Using the same procedure as 1-(4-chlorophenylsulfonyl)-7-azido-2-heptanol, 1-(4-methylphenylsulfonyl)-7-azido-2-heptanol was synthesized and purified from 4-(methylsulfonyl)toluene (853.8 mg, 5.02 mmol) yielding a colorless oil (391.1 mg, 1.26 mmol, 25 %). $^1$H-NMR (600 MHz, $CDCl_3$): δ 7.80 (2H, d, *J* = 8.4 Hz), 7.38 (2H, d, *J* = 8.4 Hz), 4.13 (1H, m), 3.41 (1H, m), 3.23 (2H, t, *J* = 6.6 Hz), 3.16 (2H, m), 1.6 ~ 1.3 (8H, m).

**O-(6-azidohexyl)-O'-succinimidyl carbonate (NHS-AZ-R1)**. Pyridine (700 µL, 8.4 mmol) was added dropwise to a stirred solution of 6-azidohexanol (608 mg, 4.2 mmol) and triphosgene (2.066 g, 6.7 mmol) in 25 mL of anhydrous THF. The resulting mixture was stirred for 15 min, gravity filtered, and concentrated to provide a crude chloroformate oil. The crude oil was dissolved in 25 mL of anhydrous THF, reacted with N-hydroxysuccinimide (1.47 g, 12.6 mmol) and pyridine (1 mL, 1.4 mmol) for 15 min. The crude mixture was concentrated, dissolved in ethyl acetate, and washed with 0.1 M HCl, water, saturated aqueous $NaHCO_3$, water, and brine, then dried over $MgSO_4$ and concentrated. Flash column chromatography on silica gel using a

gradient of ethyl acetate in hexanes of 0 – 50% yielded a colorless oil (0.7294 g, 60%). $^1$H NMR (600 MHz, $CDCl_3$) δ 4.33 (t, *J* = 6.5 Hz, 2H), 3.28 (t, *J* = 6.8 Hz, 2H), 2.84 (s, 4H), 1.84 – 1.69 (m, 2H), 1.68 – 1.52 (m, 2H), 1.43 (m, 4H).

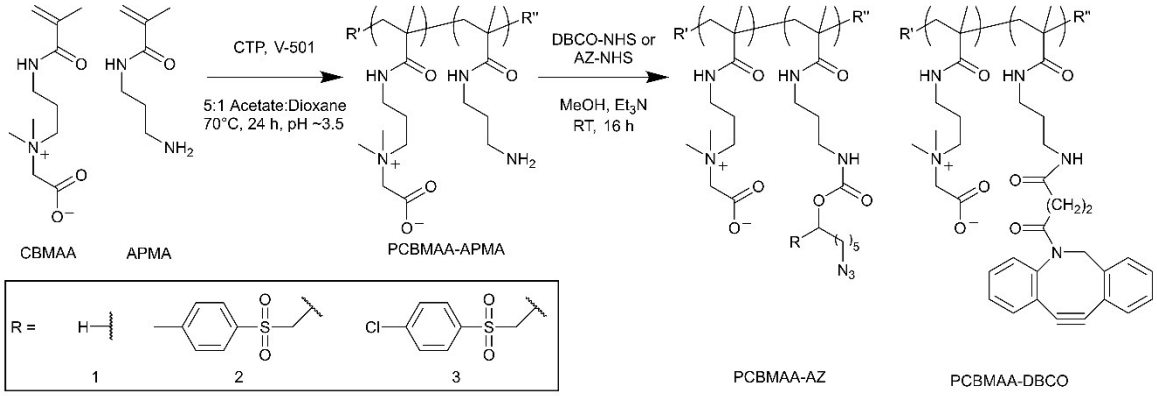
**O-[1-(4-methylphenylsulfonyl)-7-azido-2-heptyl]-O′-succinimidyl carbonate (NHS-AZ-R2).** Using the same procedure as O-[1-(4-chlorophenylsulfonyl)-7-azido-2-heptyl]-O′-succinimidyl carbonate, O-[1-(4-methylphenylsulfonyl)-7-azido-2-heptyl]-O′-succinimidyl carbonate was synthesized and purified from 1-(4-methylphenylsulfonyl)-7-azido-2-heptanol (391.1 mg, 1.26 mmol) yielding a pinkish oil (367 mg, 0.81 mmol, 65%).

**O-[1-(4-chlorophenylsulfonyl)-7-azido-2-heptyl]-O′-succinimidyl carbonate (NHS-AZ-R3)**. Pyridine (359 µL, 4.5 mmol) was added dropwise to a stirred solution of 1-(4-chlorophenylsulfonyl)-7-azido-2-heptanol (739 mg, 2.23 mmol) and triphosgene (664 mg, 2.24 mmol) in 37 mL of anhydrous THF. The resulting mixture was stirred for 15 min, then filtered and concentrated to provide the crude chloroformate as an oil. The chloroformate was dissolved in 37 mL of dry THF and treated reacted with N-hydroxysuccinimide (769 mg, 6.83 mmol) and pyridine (557 µL, 6.9 mmol) for 15 min. The mixture was then concentrated, and the residue was dissolved in ethyl acetate. After washing with 0.1 M HCl, water, saturated aq. $NaHCO_3$, water, and brine, the solution was dried over $MgSO_4$, filtered, and concentrated. The crude succinimidyl carbonate was purified by flash column chromatography on silica gel using a gradient of ethyl acetate in hexane of 0 - 50 % to yield the product as a yellow oil that crystallizes on standing (750 mg, 1.59 mmol, 72%).

**Cell encapsulation in P(EG)$_{4-5}$MA hydrogels**. 60 µL 5 wt% P(EG)$_{4-5}$MA hydrogels with 20,000 encapsulated NIH 3T3 fibroblasts were cultured in DMEM-F12 media with 10% CBS. Hydrogels were formed by mixing equal volumes of P(EG)$_{4-5}$MA-AZ and P(EG)$_{4-5}$MA-DBCO PBS solutions that each contained 10,000 cells and incubating at 37 °C for 1 h. As a non-cytotoxic control, 20,000 cells were also encapsulated in 1 wt% agarose hydrogels by mixing equal volumes of

cell suspension with a 2 wt% agarose solution in PBS. Agarose gels were placed at 4 °C for 15 min and incubated at 37 °C for 45 min. 200 µL of media was then added on top of each gel and incubated at 37 °C 5% $CO_2$. After 20 h, the media was removed from gel surfaces and replaced with media containing 10% PrestoBlue reagent. After 1 h, fluorescence was measured using a BioTek Cytation 5 plate reader ($\lambda_{ex}$ = 560 nm; $\lambda_{em.}$ = 590 nm) and normalized to the non-cytotoxic agarose control.

## B. Supplemental Figures




**Scheme S1. Synthesis of PCBMAA-AZ and PCBMAA-DBCO copolymers for in situ crosslinking.** PCBMAA copolymers were synthesized by RAFT polymerization for subsequent derivatization with NHS-AZ derivatives and NHS-DBCO. 3 different PCBMAA-AZ copolymers were synthesized with a non-degradable linker (R1), and 2 degradable linkers with EWGs (R2 = 4-methylphenyl sulfone, and R3 = 4-chlorophenyl sulfone).

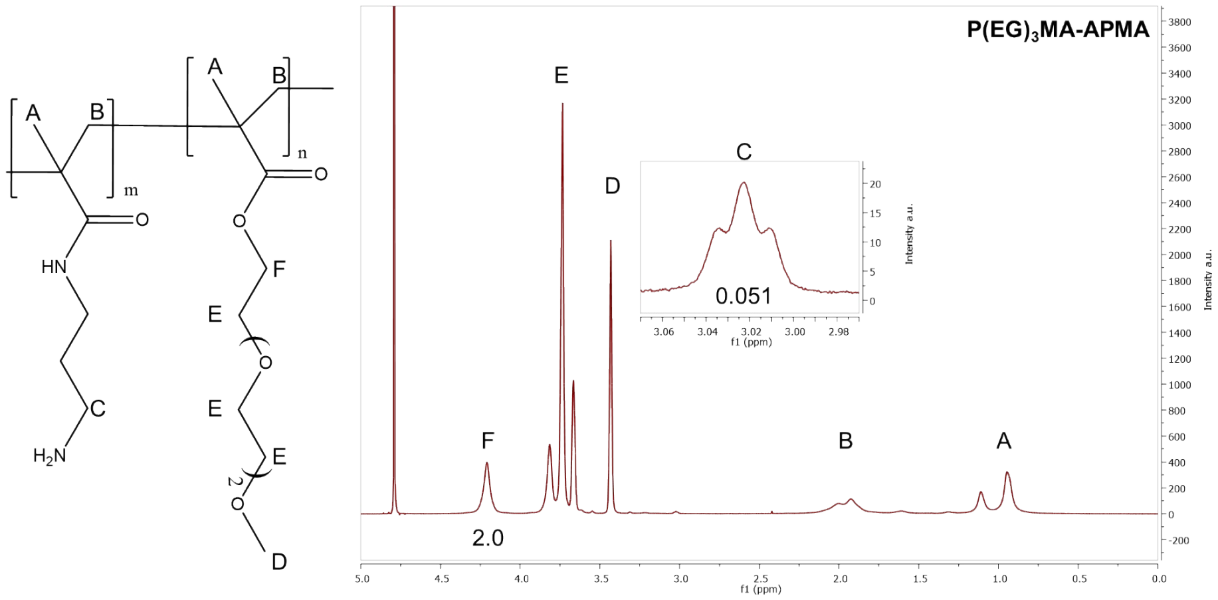



**Figure S1.** $^{1}H$ NMR $P(EG)_3MA$-APMA in $D_2O$.

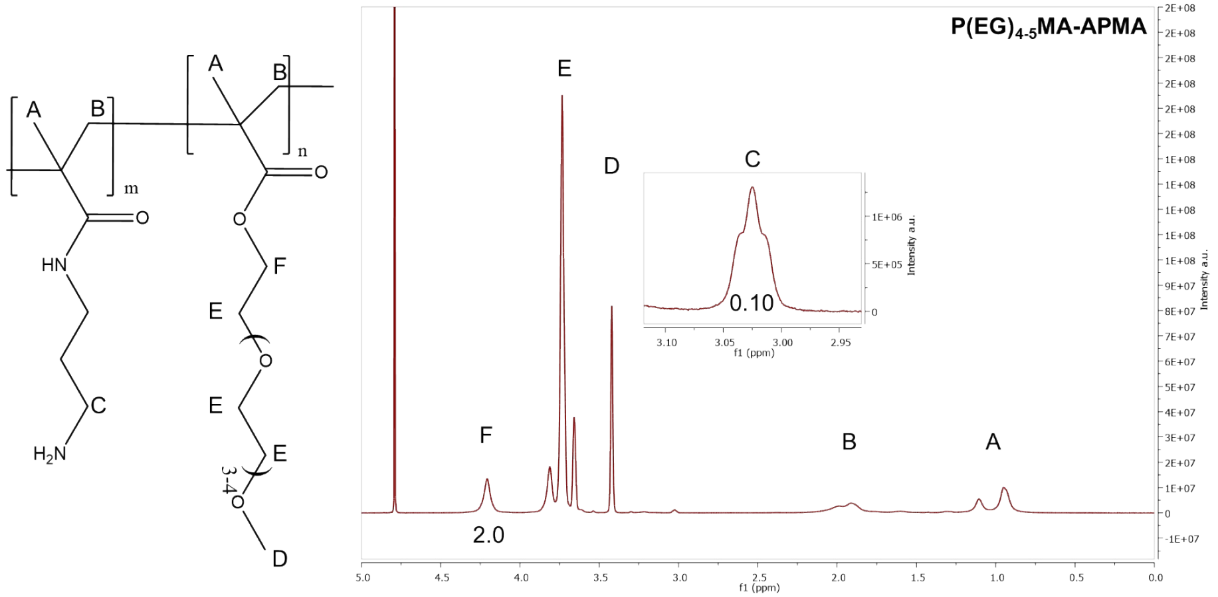



**Figure S2.** $^{1}H$ NMR $P(EG)_{4\text{-}5}MA$-APMA in $D_2O$.

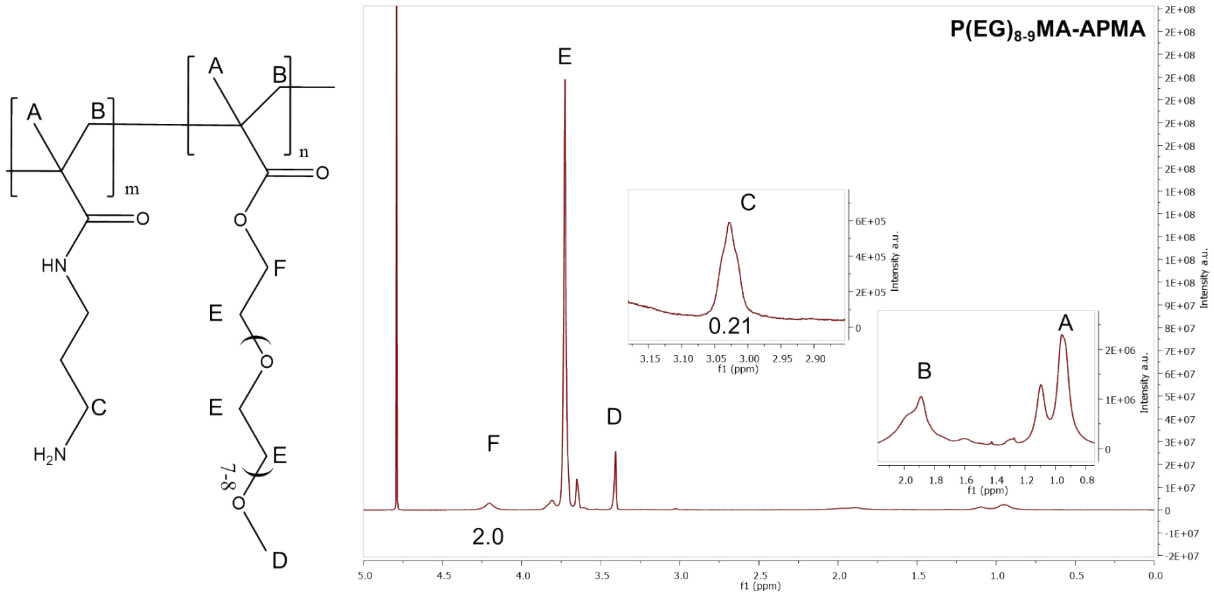



**Figure S3**. $^{1}H$ NMR $P(EG)_{8\text{-}9}MA\text{-}APMA$ in $D_2O$.

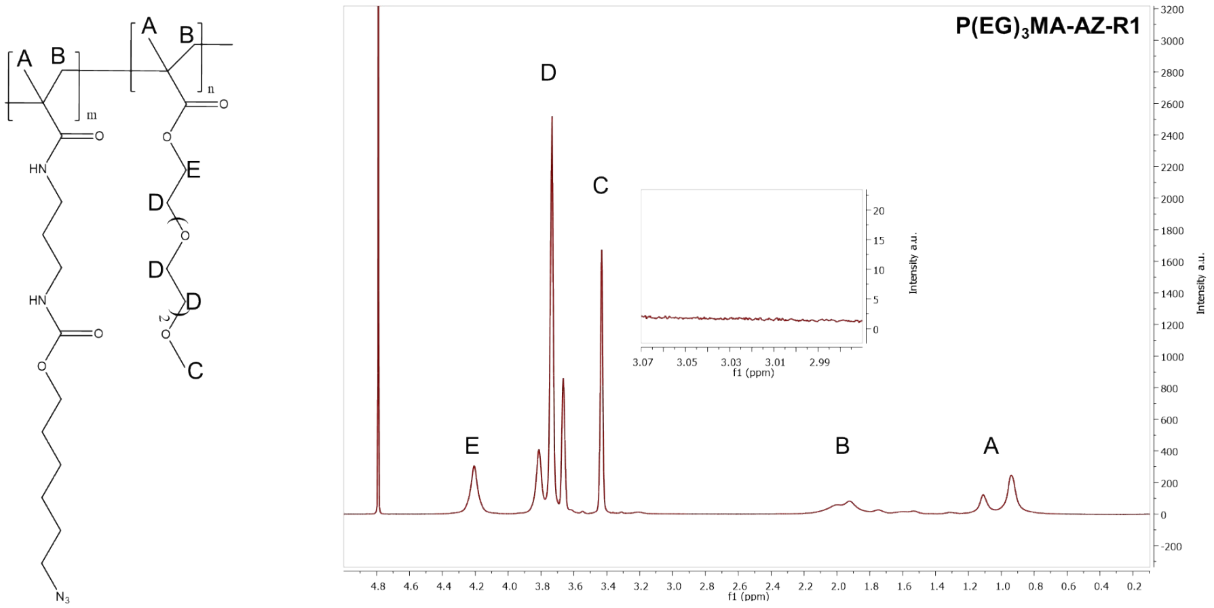



**Figure S4**. $^{1}H$ NMR $P(EG)_{3}MA\text{-}AZ\text{-}R1$ in $D_2O$.

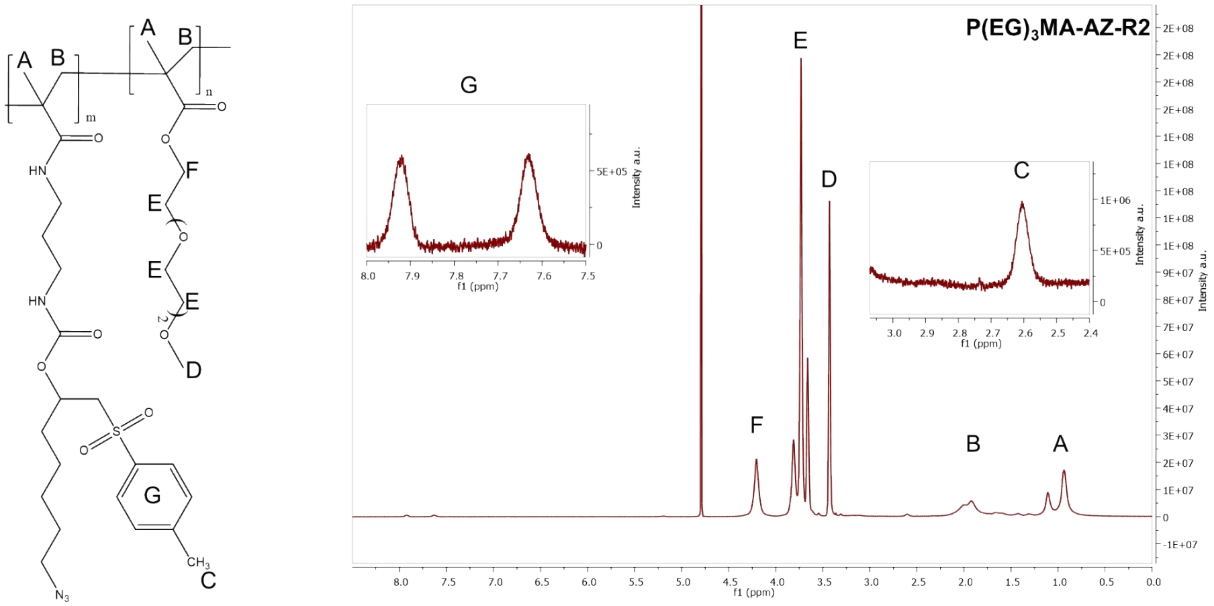



**Figure S5**. $^{1}H$ NMR $P(EG)_3MA$-AZ-R2 in $D_2O$.

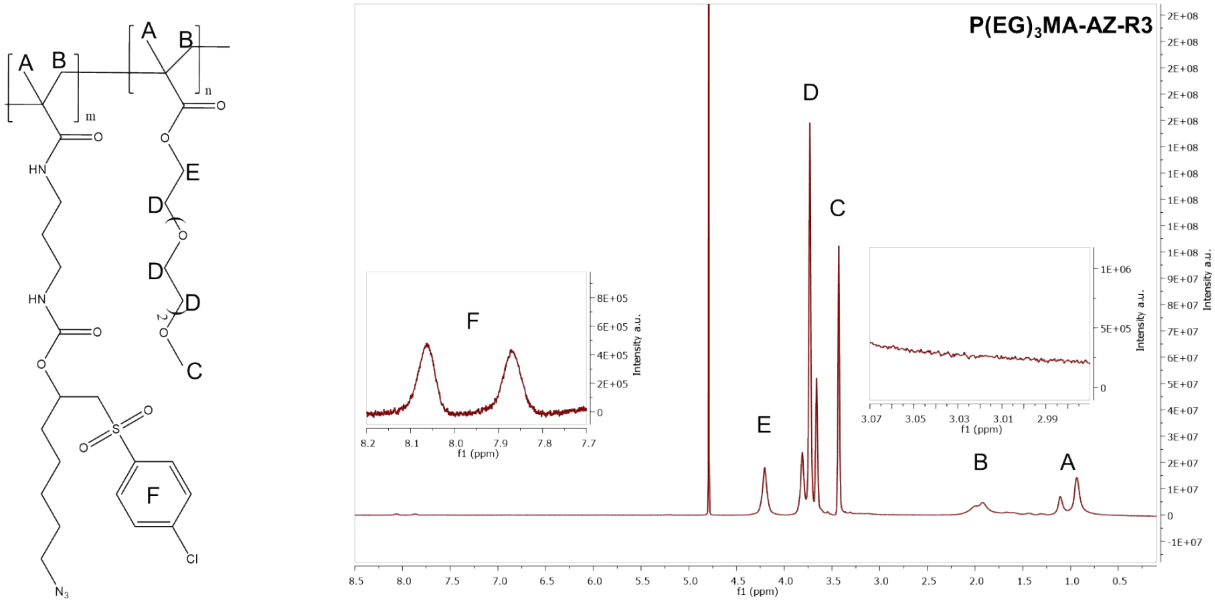



**Figure S6**. $^{1}H$ NMR $P(EG)_3MA$-AZ-R3 in $D_2O$.

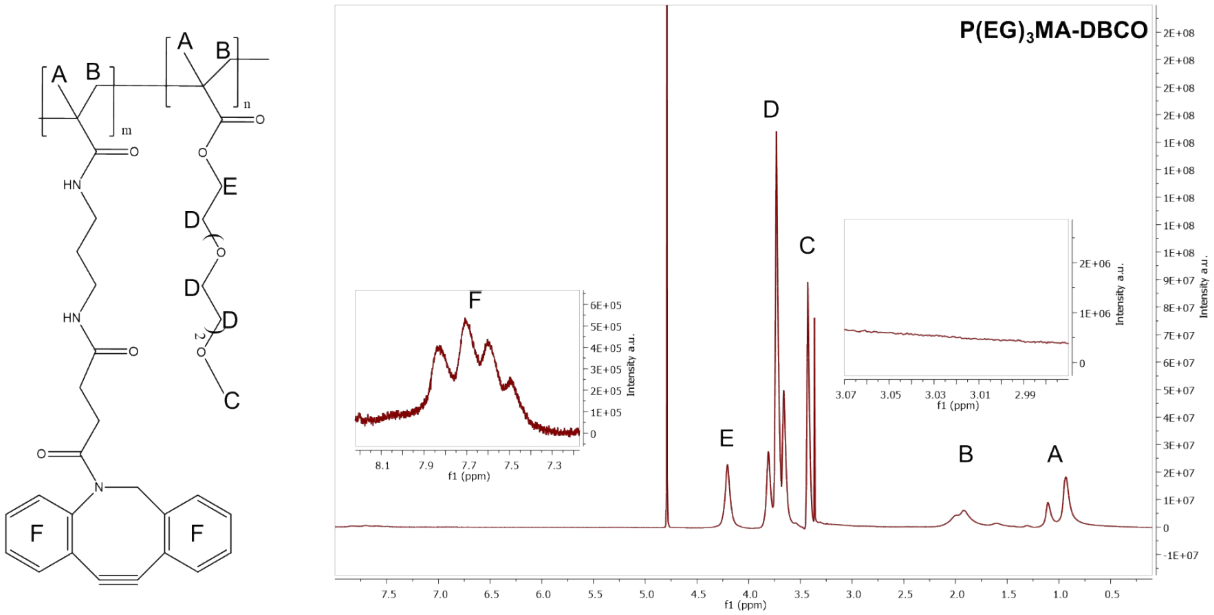



**Figure S7**. $^{1}H$ NMR $P(EG)_3MA$-DBCO in $D_2O$.

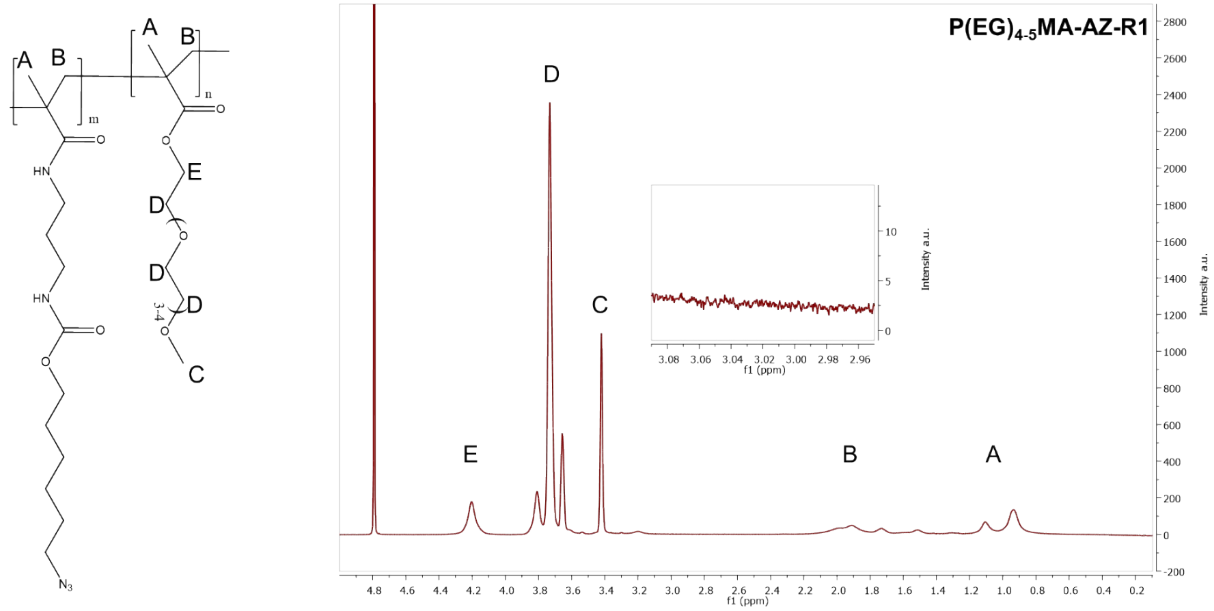



**Figure S8**. $^{1}H$ NMR $P(EG)_{4\text{-}5}MA$-AZ-R1 in $D_2O$.

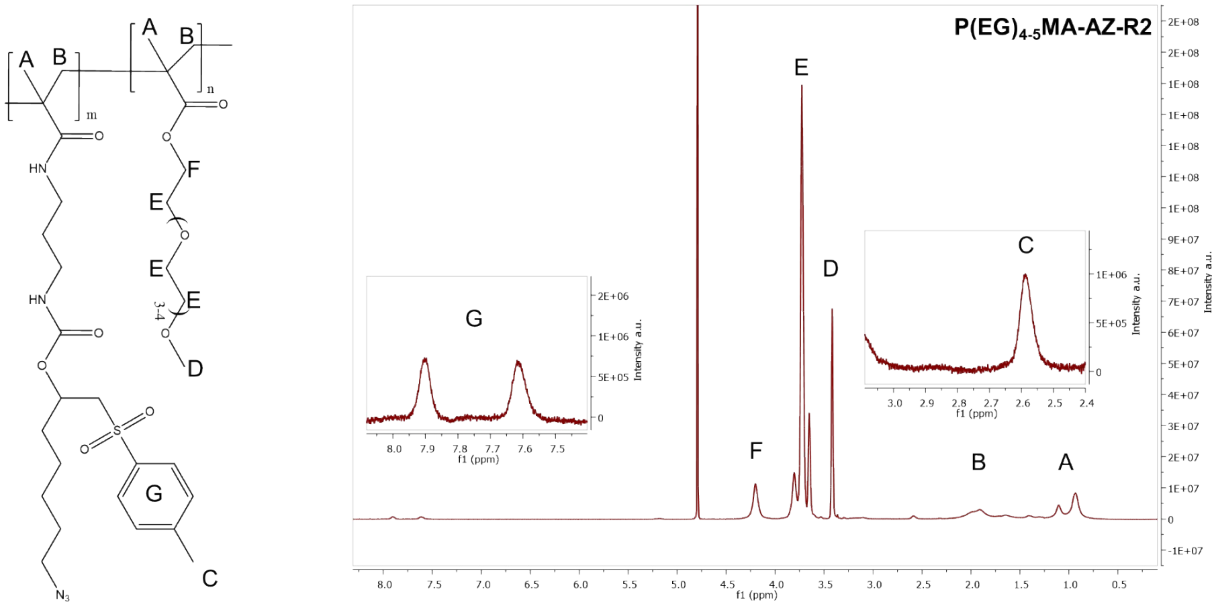



**Figure S9**. $^{1}$H NMR P(EG)$_{4\text{-}5}$MA-AZ-R2 in $D_2O$.

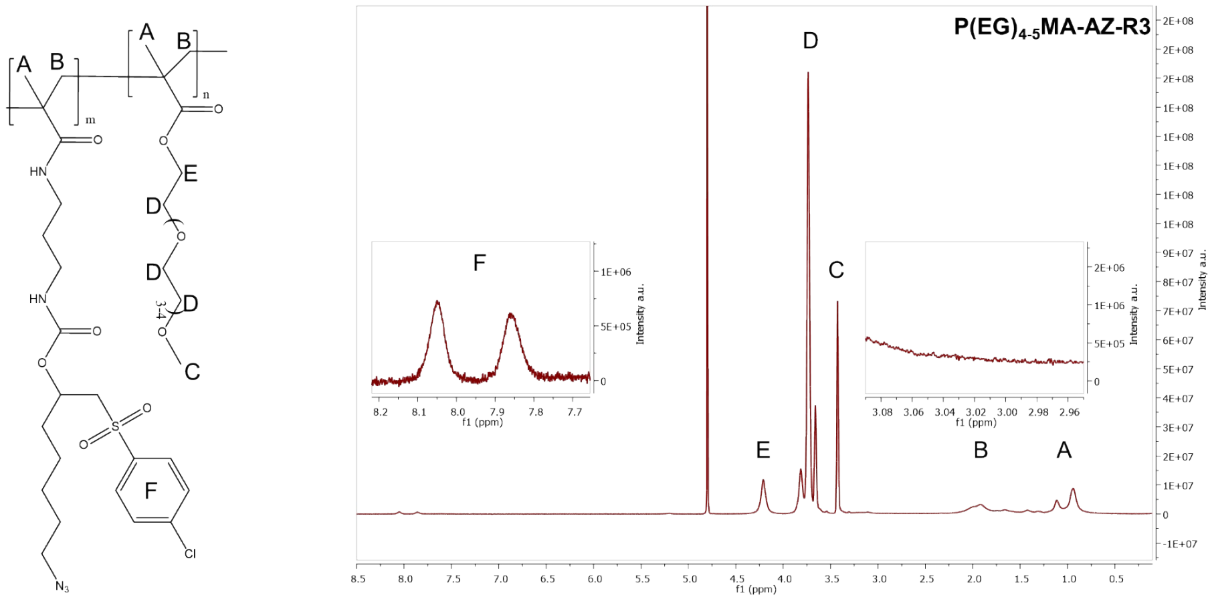



**Figure S10**. $^{1}$H NMR P(EG)$_{4\text{-}5}$MA-AZ-R3 in $D_2O$.

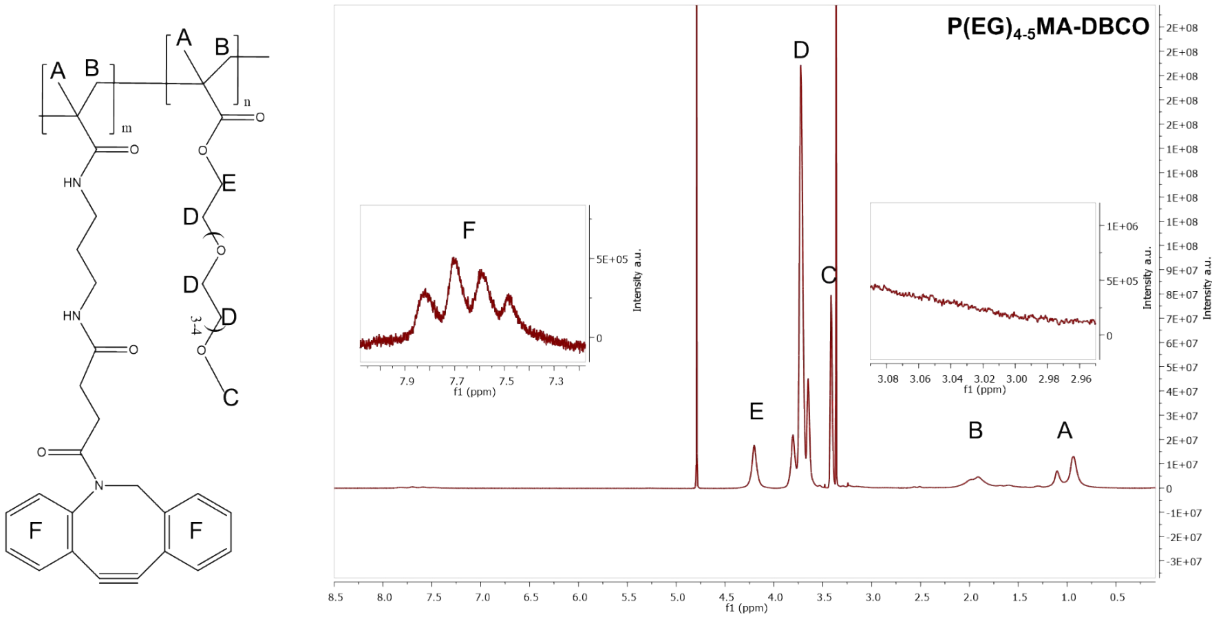



**Figure S11**. $^1$H NMR P(EG)$_{4\text{-}5}$MA-DBCO in $D_2O$. Sharp peak at ~3.4 ppm is residual MeOH solvent.

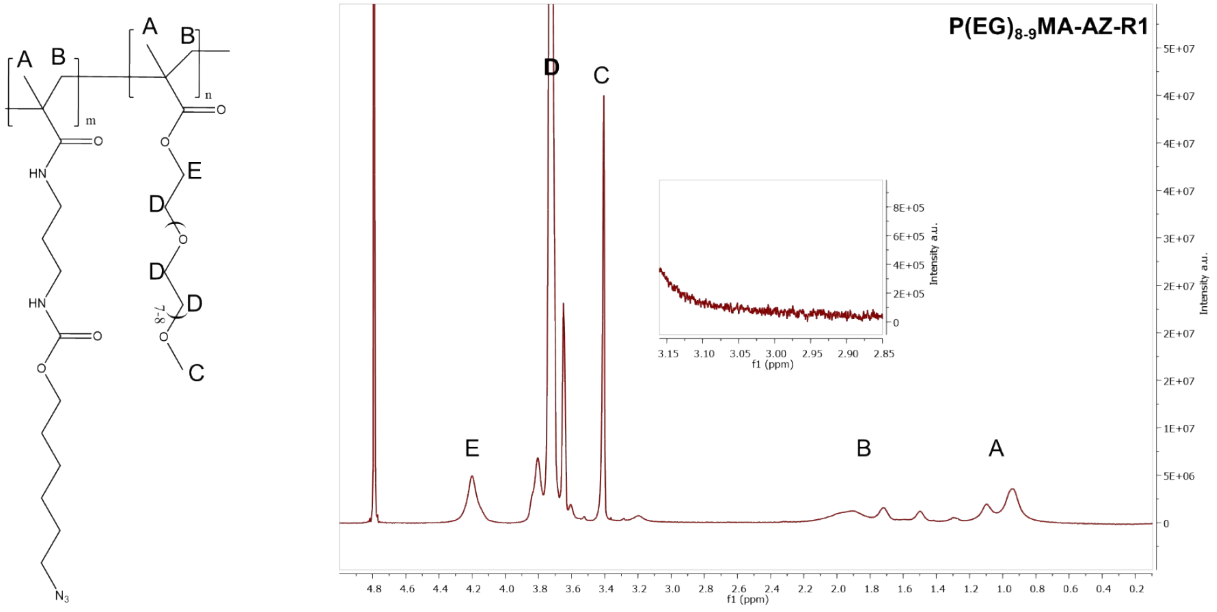



**Figure S12**. $^1$H NMR P(EG)$_{8\text{-}9}$MA-AZ-R1 in $D_2O$. Peak D set off-scale to make other peaks visible.

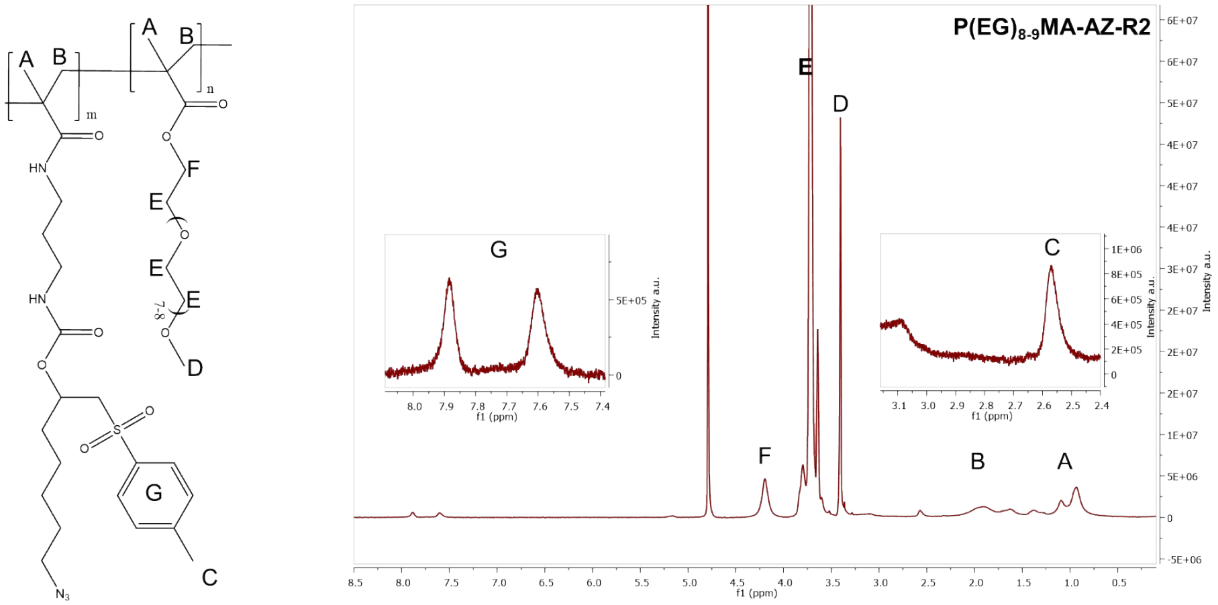



**Figure S13**. $^1$H NMR P(EG)$_{8\text{-}9}$MA-AZ-R2 in $D_2O$. Peak E set off-scale to make other peaks visible.

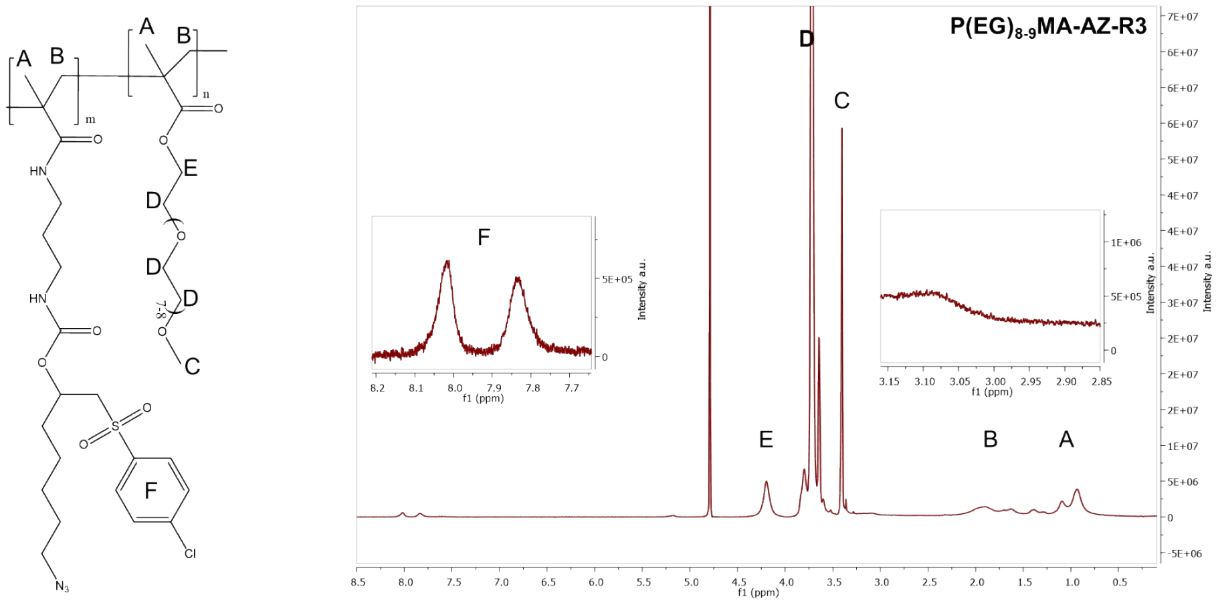



**Figure S14**. $^1$H NMR P(EG)$_{8\text{-}9}$MA-AZ-R3 in $D_2O$. Peak D set off-scale to make other peaks visible.

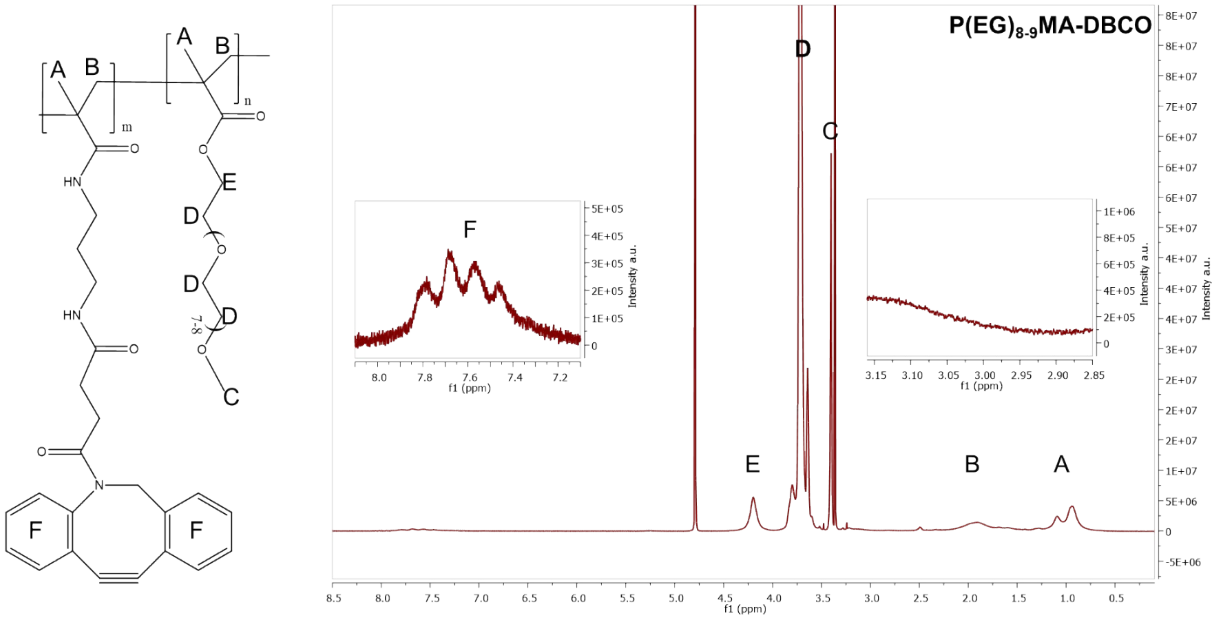



**Figure S15**. $^1$H NMR P(EG)$_{8\text{-}9}$MA-DBCO in $D_2O$. Peak D set off-scale to make other peaks visible. Sharp peak at ~3.4 ppm is residual MeOH solvent.

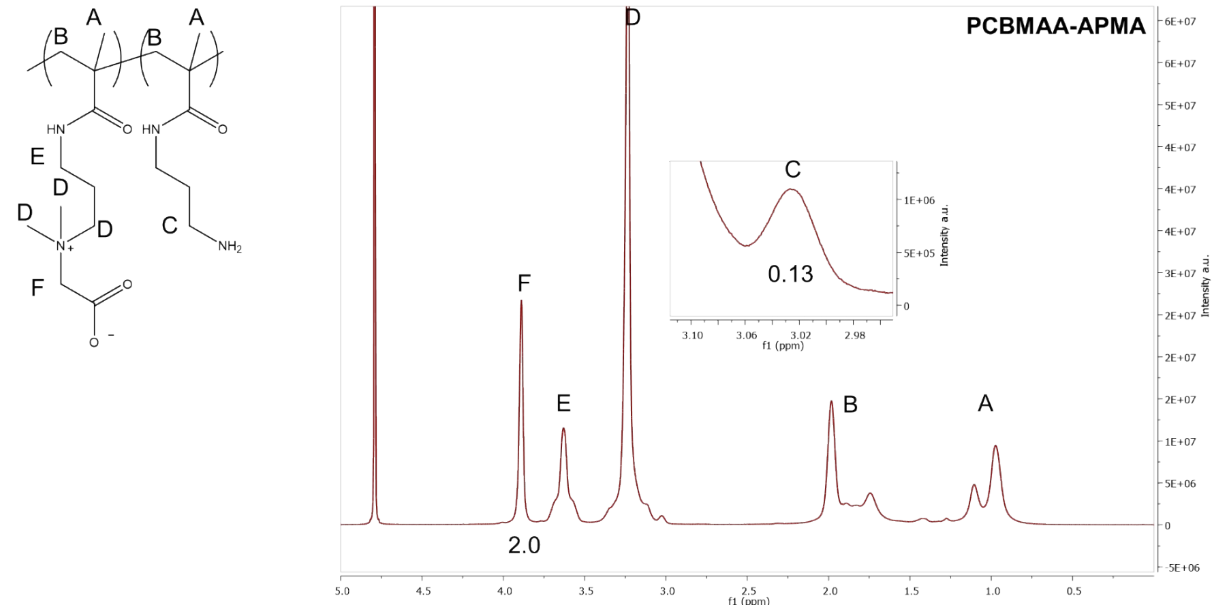



**Figure S16**. $^1$H NMR PCBMAA-APMA in $D_2O$.

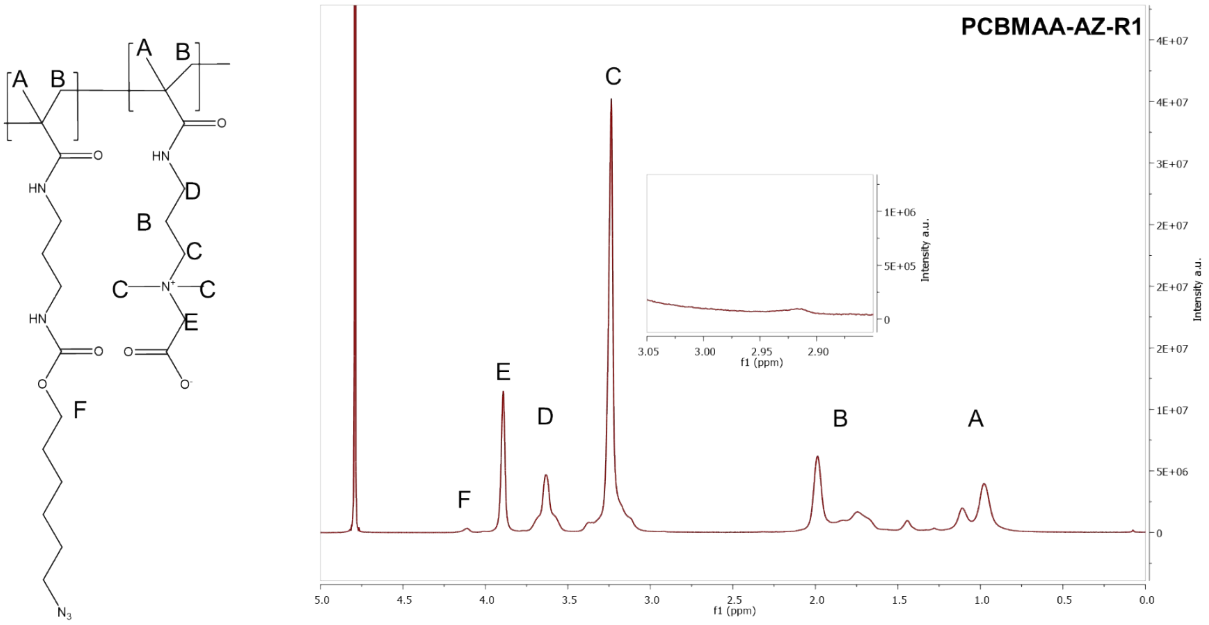



**Figure S17**. $^{1}H$ NMR PCBMAA-AZ-R1 in $D_2O$.

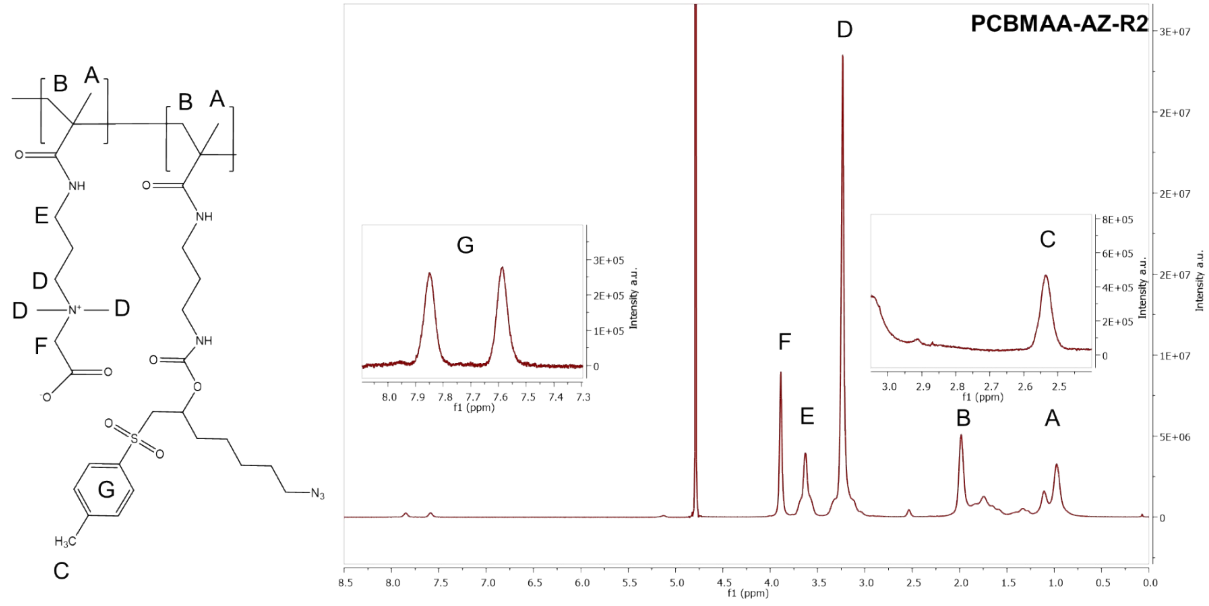



**Figure S18**. $^{1}H$ NMR PCBMAA-AZ-R2 in $D_2O$.

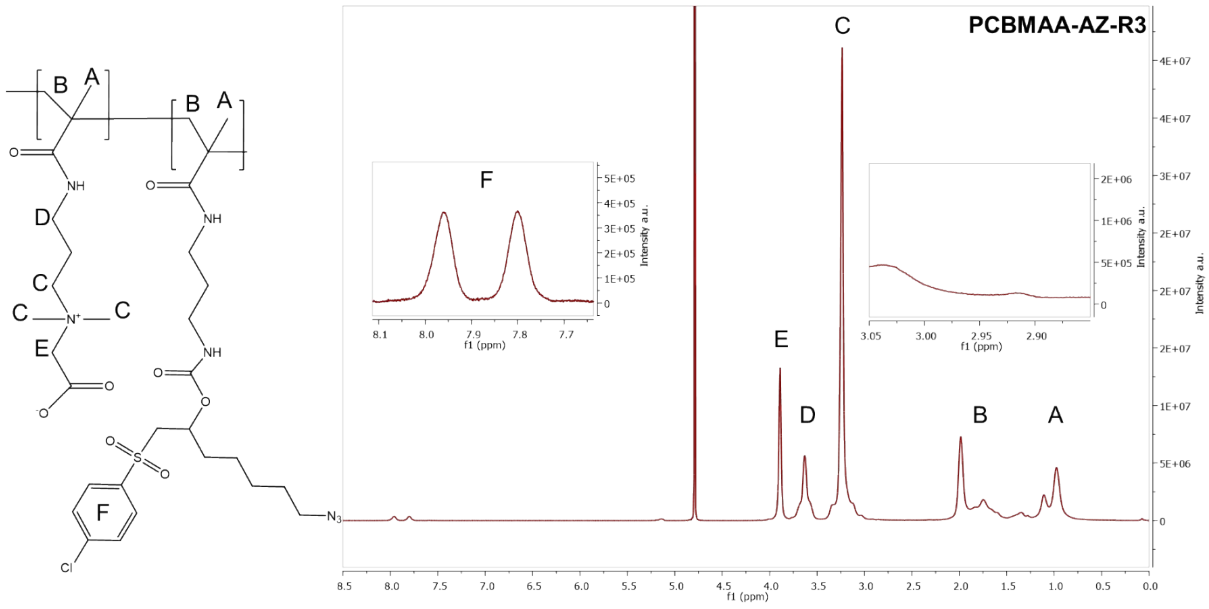



**Figure S19**. $^1$H NMR PCBMAA-AZ-R3 in $D_2O$.

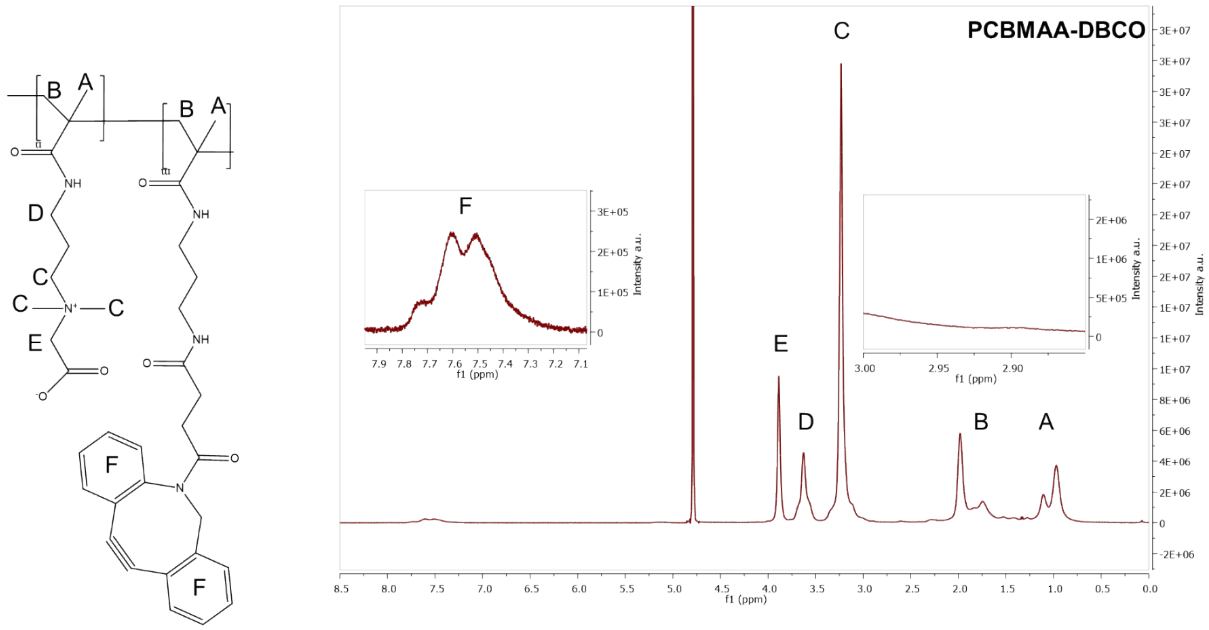



**Figure S20**. $^1$H NMR PCBMAA-DBCO in $D_2O$.

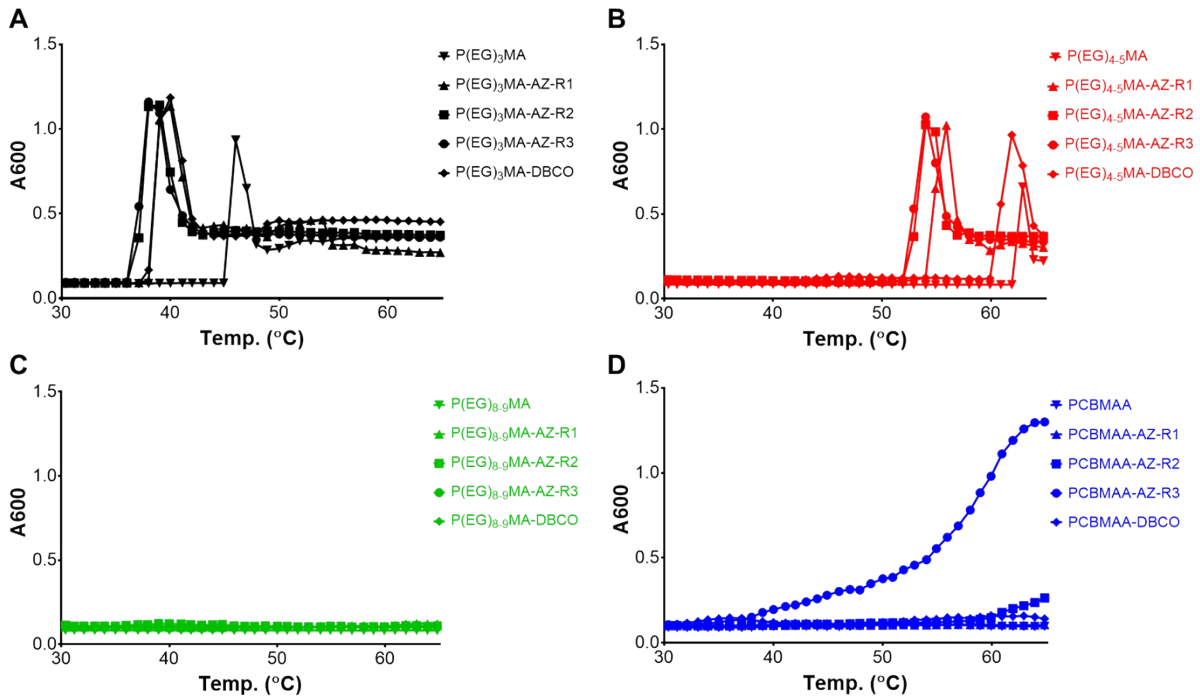



**Figure S21**. **LCST of P(EG)$_x$MA and PCBMAA copolymers**. Turbidity (600 nm) of 25 g L$^{-1}$ polymer solutions in PBS was measured from 30 - 65°C. Onset of turbidity was defined as the LCST. Note that the increased turbidity of PCBMAA-AZ-R3 is due to hydrolysis of the degradable linker (R3) leading to an insoluble hydrophobic by-product. In hydrogel formation, all hydrophobic groups will remain attached to a polymer, preventing insoluble products from forming.

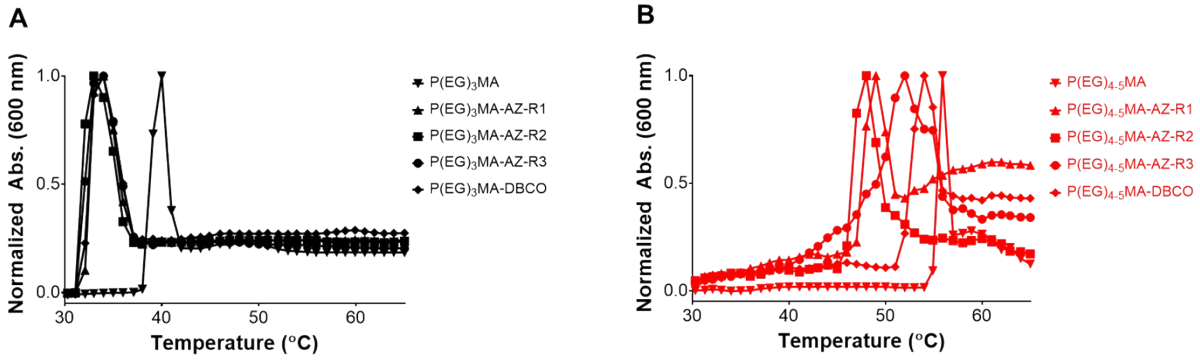



**Figure S22. LCST of P(EG)$_3$MA and P(EG)$_{4\text{-}5}$ polymers**. Turbidity (600 nm) of 25 g L$^{-1}$ polymer solutions in 0.1 M borax was measured from 30 - 65°C. Onset of turbidity was taken as the LCST.

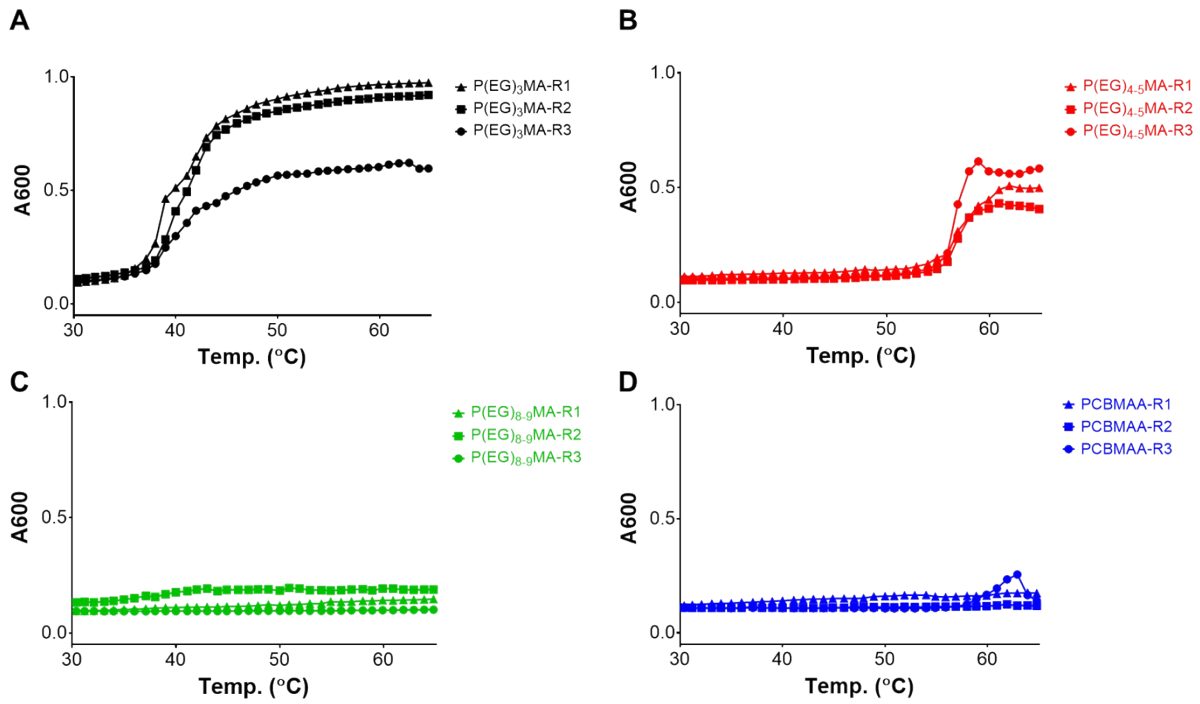



**Figure S23**. **Cloud point of P(EG)$_x$MA and PCBMAA hydrogels**. Turbidity (600 nm) of 5 wt % hydrogels in PBS was measured from 30 - 65°C. Onset of turbidity was defined as the cloud point.

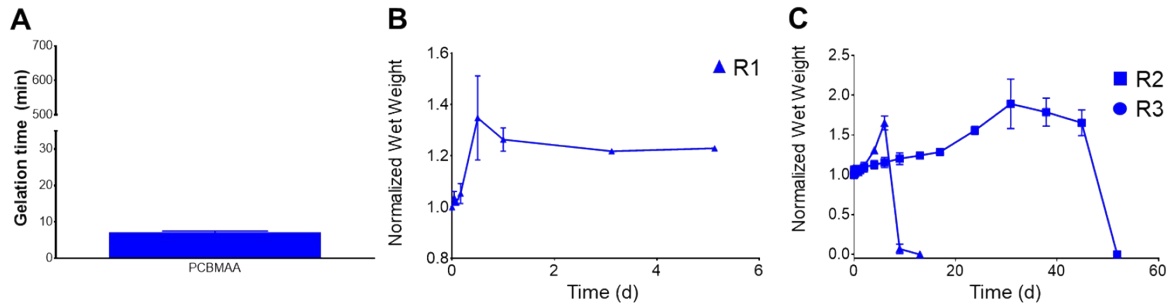



**Figure S24. Characterization of PCBMAA hydrogels: gelation time, swelling and degradation. A**) Gelation time of PCBMAA-R1 hydrogels (6 mol% crosslinker; 5 wt %) determined by gravitational flow analysis (mean ± standard deviation, n = 6). **B**) Equilibrium swelling of PCBMAA-R1 gels. After overnight gelation, hydrogels (5 wt %) were submerged in PBS and their wet weight was determined at specific time points (mean ± standard deviation, n = 3). **C**) Tunable degradation of PCBMAA gels. Degradation of PCBMAA gels (100 µL, 5 wt %) with R2 (4-methylphenyl sulfone) and R3 (4-chlorophenyl sulfone) crosslinkers was followed over time in pH 7.4 PBS at 37°C (mean ± standard deviation, n = 3).

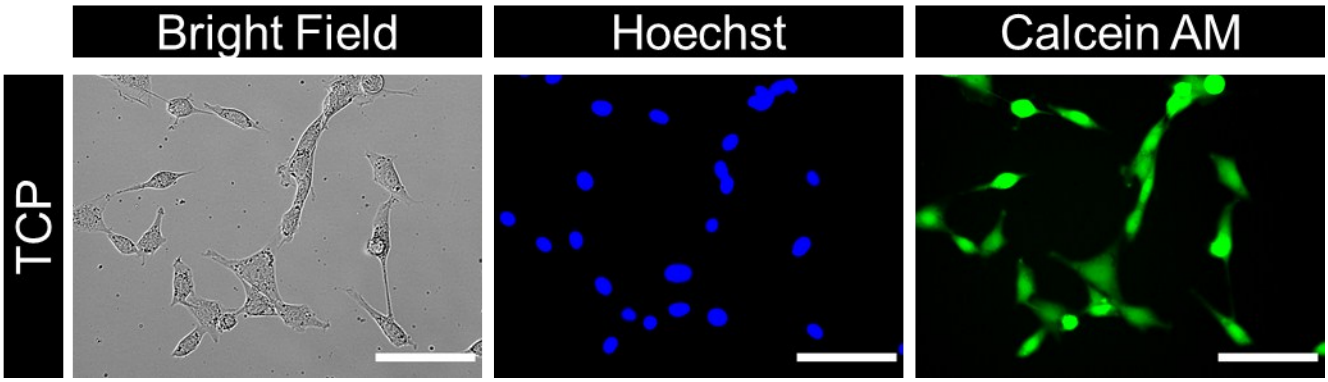



**Figure S25. Cell adhesion to tissue culture plastic (TCP).** NIH 3T3 mouse fibroblasts (5000 cells per well) were seeded on TCP and incubated for 24 h at 37°C. Calcein AM and Hoescht was then added and incubated for 15 min at 37°C. Wells were then washed 3 times with PBS to remove unadhered cells prior to imaging using a BioTek Cytation 5 cell imager. Scale bars are 100 μm.

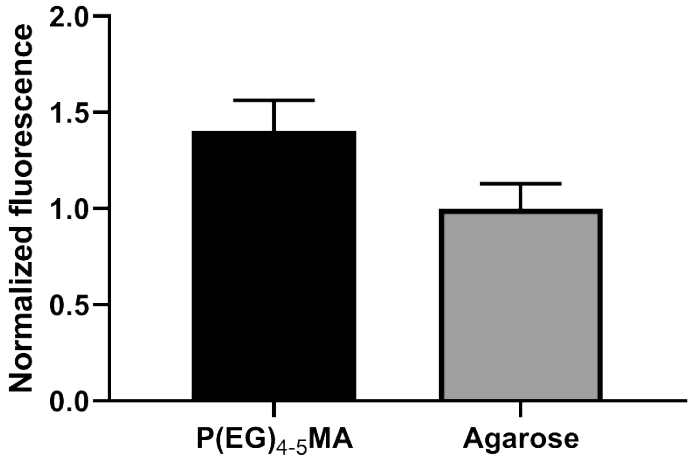



**Figure S26. Encapsulated cells remained viable in $P(EG)_{4\text{-}5}MA$ gels.** 20,000 NIH 3T3 fibroblasts were encapsulated in 5 wt% $P(EG)_{4\text{-}5}MA$ and non-cytotoxic 1 wt% agarose hydrogels. After incubating for 20 h, cell viability was assessed using the fluorescent PrestoBlue assay. Cells encapsulated in $P(EG)_{4\text{-}5}MA$ gels demonstrated higher viability than cells in agarose gels ($p < 0.03$; mean ± standard deviation, $n = 3$), indicating the $P(EG)_{4\text{-}5}MA$ crosslinking is non-cytotoxic.