

Thermoresponsive Fluorinated Polyacrylamides with Low Cytotoxicity

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Experimental

Materials. Acryloyl chloride (96%) was purchased from MERCK. 2-(Dodecylthiocarbonothioylthio)-2-methylpropionic acid (DMP, 98%), N,N-dimethylformamide (DMF, 99.8%), 2-Fluoroethylamine hydrochloride (90%), Triethylamine (TEA, 99%), and 1,3,5-Trioxane (99%) were purchased from Aldrich and used as received. 2,2'-Azobisisobutyronitrile (AIBN, Aldrich, 98%) was recrystallized from ethanol. 2,2-Difluoroethylamine (97%) was purchased from Oakwood and used as received. 2,2,2-Trifluoroethyl amine (97%) was purchased from TCI and used as received. A549 human airway epithelial cells were grown in minimum essential medium (MEM, Gibco/BRL, Rockville, MD, USA)

containing 10 % fetal bovine serum and 1 % penicillin/streptomycin (Gibco/BRL). The cells were placed in the wells of 96-well plates at a density of 1×10^4 cells/well and the plates were incubated overnight under normal culture conditions (37 °C and 5 % CO₂). After 1 hr adjustment with serum-free media, polymers reconstituted in the same serum-free media at varying concentrations were added to the A549 cells. After a 24-hr treatment, A549 cell viability was assessed by the MTT cell viability assay.

Characterization. ¹H NMR spectra were collected in DMF-d₇ and CDCl₃ on a Bruker avance 300 MHz NMR spectrometer. The apparent molecular weights and molecular weight distributions were measured by GPC (Agilent technologies 1200 series) using a PMMA standard, with DMF as the eluent at 30 °C and a flow rate of 1.00 mL/min. UV-vis spectra were recorded using an OPTIZEN 3220 UV-vis spectrophotometer equipped with a digital temperature controller. A 650 nm wavelength was used to determine LCST. The temperature range was from 25 to 80 °C with a heating and cooling rate of 1 °C/min. The cloud point was defined as the middle point of the transmittance change.

Synthesis.

N-(2-fluoroethyl)acrylamide (M1F). 2-Fluoroethylamine hydrochloride (10 g, 102 mmol) and distilled water (200 mL) were added to round-bottomed flask. The solution was basified with KOH and extracted with dichloromethane. The resulting solution was dried over MgSO₄ and a few concentrated to give volatile colorless oil. The product was added to round-bottomed flask in the presence of TEA (17 mL, 122 mmol). The flask was immersed into an ice-water bath. Acryloyl chloride (7.7 mL, 122 mmol) in 10 mL dichloromethane was added into the mixture within 10 min. The reaction mixture was allowed to stir for overnight at room temperature, allowing insoluble salt to precipitate out of the solution. After filtration, the solvent was evaporated. The product was purified by column chromatography using Hexane-EA (1:2) as an eluent to give **M1F** as pale yellow liquid (2.3 g, 20 %).

N-(2,2-difluoroethyl)acrylamide (M2F). 2,2-difluoroethylamine (4.99 g, 61.5 mmol), TEA (10.3 mL, 73.8 mmol), and THF (50 mL) were added to round-bottomed flask and immersed into an ice-water bath. Acryloyl chloride (6.0 mL, 73.8 mmol) in 10 mL THF was added dropwise into the mixture within 10 min. The reaction mixture was allowed to stir for overnight at room temperature, allowing insoluble salt to precipitate out of solution. After filtration, the solvent was evaporated. The product was purified by column chromatography using Hexane- EA (1:2) as an eluent to give **M2F** as white crystal solid (4.2 g, 50 %).

N-(2,2,2-trifluoroethyl)acrylamide (M3F). 2,2,2-trifluoroethylamine (4.85 mL, 61.5 mmol), TEA (10.3 mL, 73.8 mmol), and THF (50 mL) were added to round-bottomed flask and immersed into an ice-water bath. Acryloyl chloride (6.0 mL, 73.8 mmol) in 10 mL THF was added dropwise into the mixture within 10 min. The reaction mixture was allowed to stir for overnight at room temperature, allowing insoluble salt to precipitate out of solution. After filtration, the solvent was evaporated. The product was purified by column chromatography using Hexane- EA (1:2) as an eluent to give **M3F** as white crystal solid (4.9 g, 53 %).

poly[N-(2,2-difluoroethyl)acrylamide] (P2F) Polymerization of **M2F** was conducted at 80 °C under a nitrogen atmosphere, employing 2-(Dodecylthiocarbonothioylthio)-2-methylpropionic acid (DMP) as the RAFT CTA and AIBN as the primary radical source. A typical RAFT polymerization procedure was as follows. **M2F** (1.35 g, 20.0 mmol), DMP (18.2 mg, 0.1 mmol), 1,3,5-trioxane (45.0 mg, 1.0 mmol, internal standard), AIBN (0.41 mg, 0.005 mmol), and DMF (4.5 mL) were sealed in a 10 mL Schlenk flask equipped with a magnetic stir bar. The solution was purged with argon for 20 min, and the reaction flask was placed in a preheated oil bath at 80 °C. Samples were removed periodically by syringe to determine molecular weight and polydispersity index (PDI) by gel permeation chromatography (GPC) and monomer conversion by ¹H NMR spectroscopy. The polymerization was quenched by cooling in liquid nitrogen and exposing the solution to air. The solution was concentrated

under vacuum, and the polymer was precipitated into cold ether. The polymer was redissolved in DMF and reprecipitated into cold ether and dried under vacuum at room temperature for 24 h.

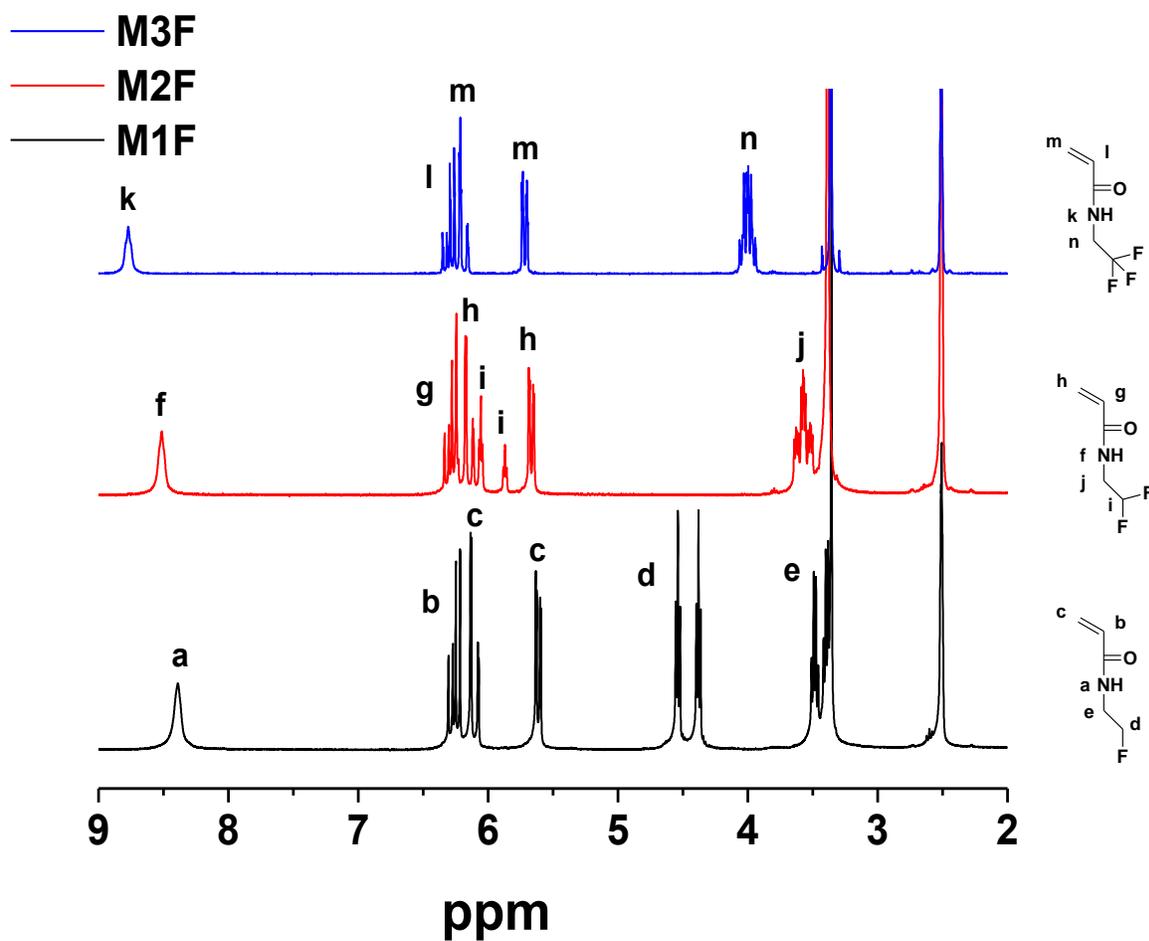


Figure S1. ¹H NMR spectra of M1F, M2F, and M3F.

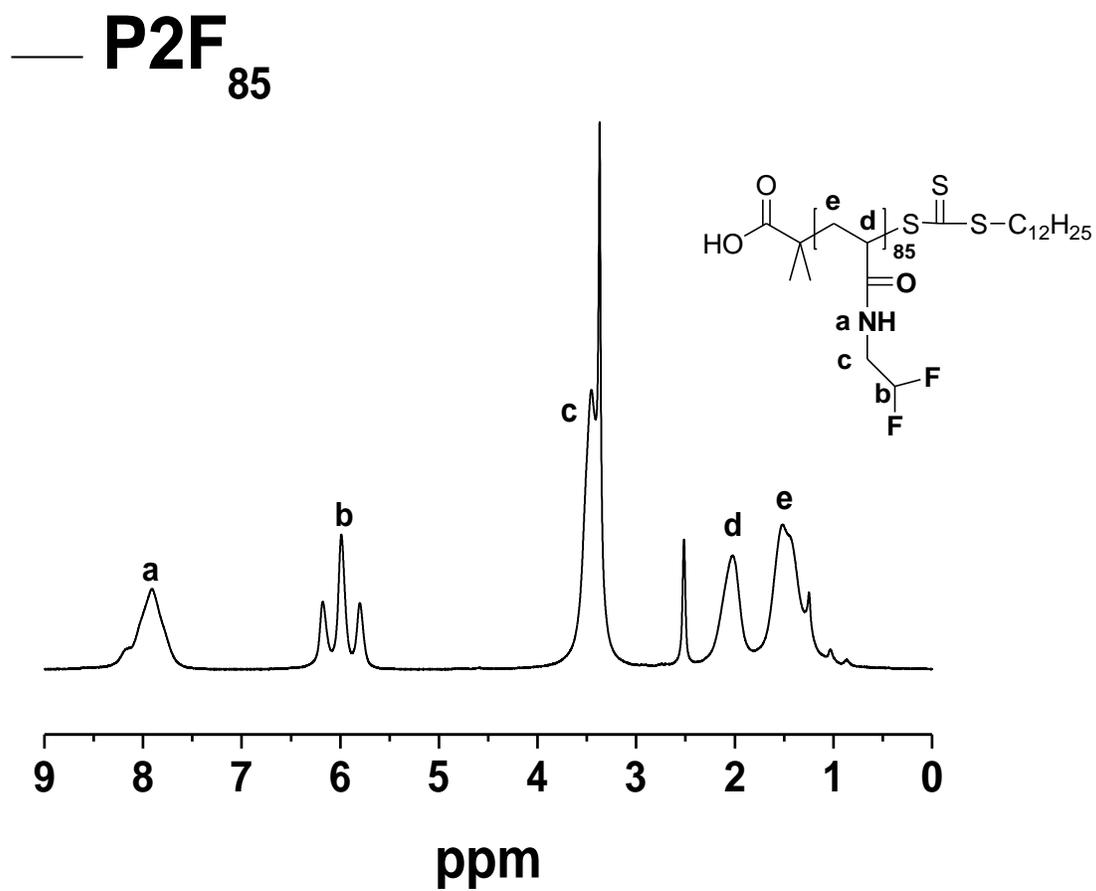


Figure S2. ¹H NMR spectra of P2F₈₅ synthesized by RAFT polymerization.

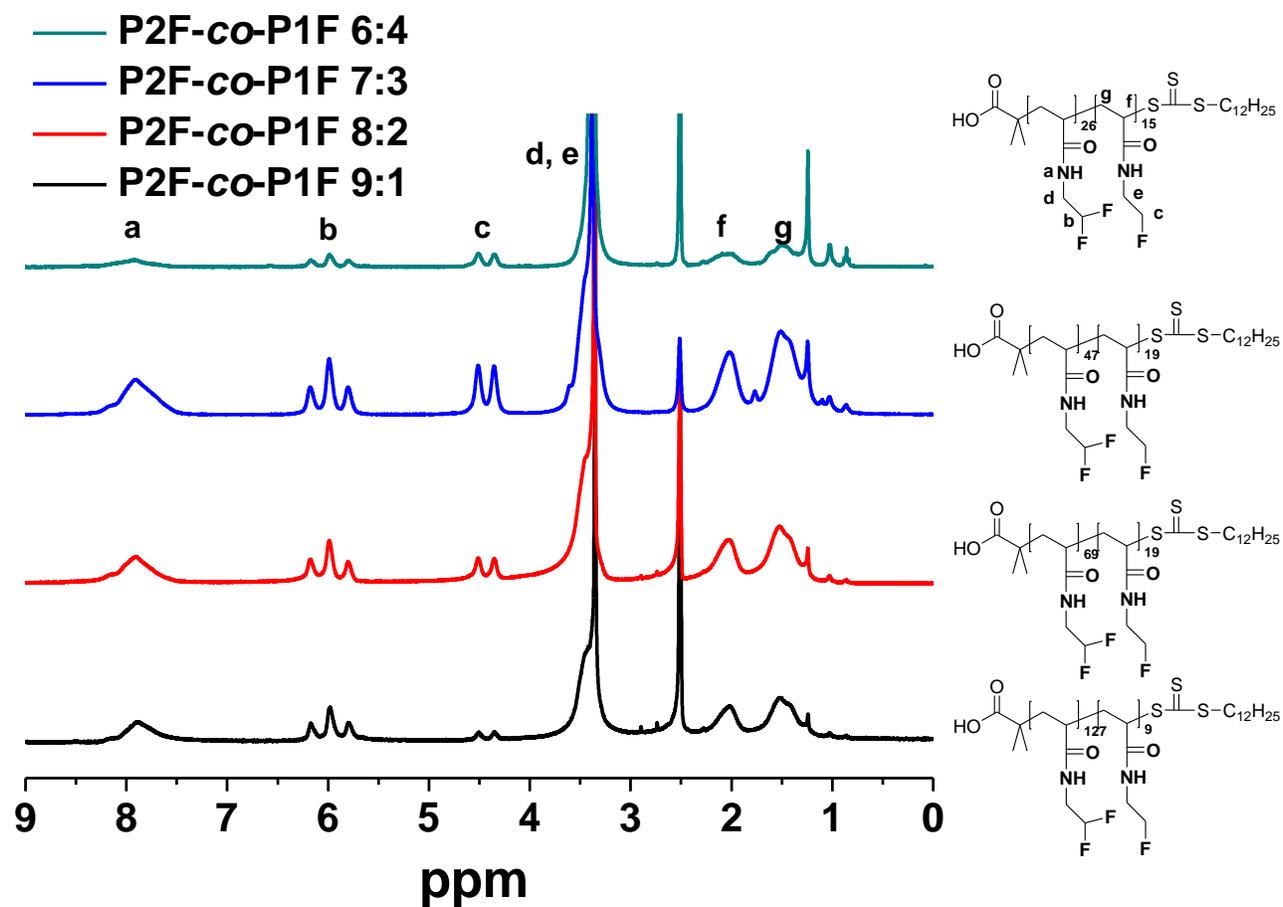


Figure S3. ¹H NMR spectra of a series of P2F-co-P1F copolymer synthesized by RAFT polymerization with different initial feed ratios of M2F and M1F.

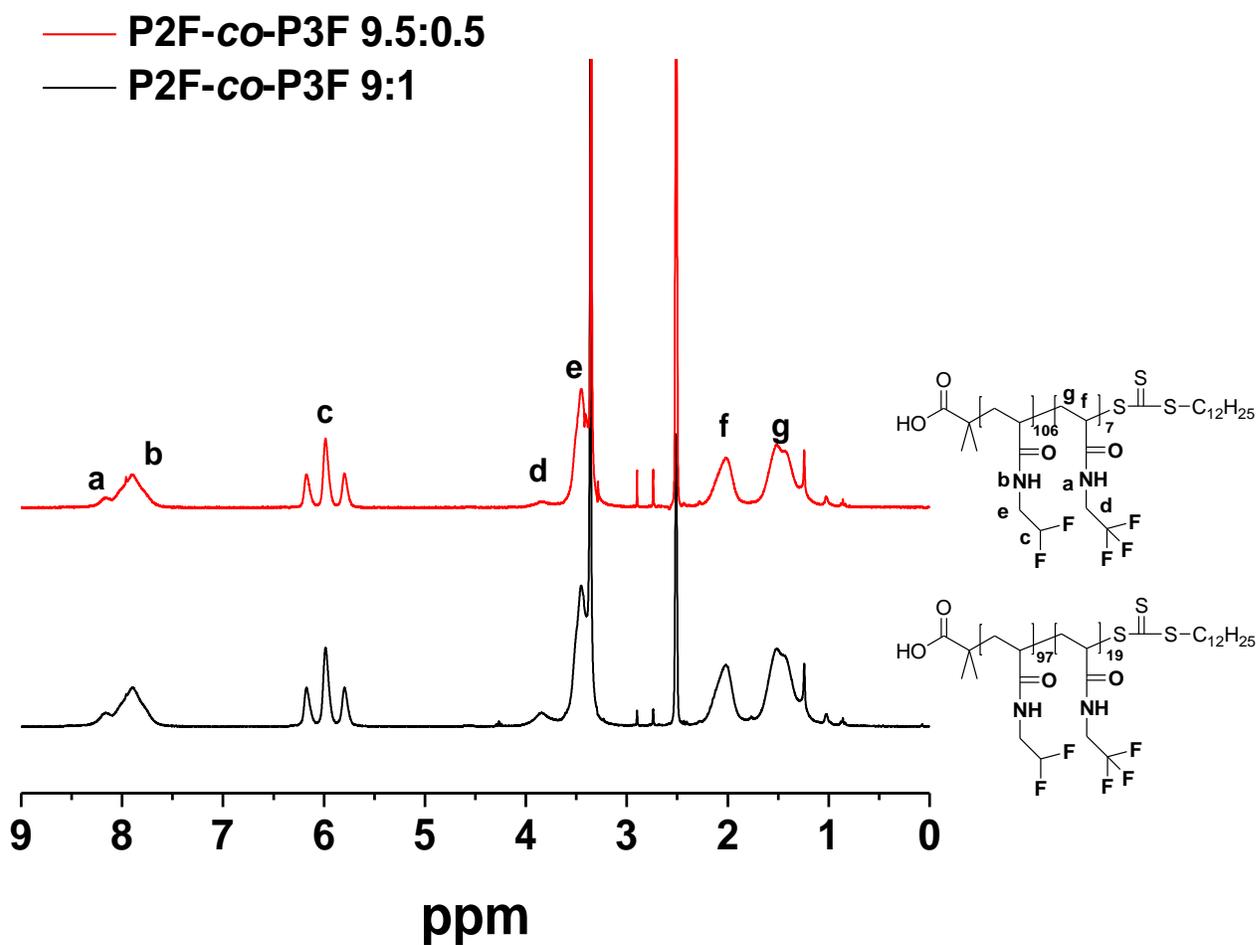


Figure S4. ^1H NMR spectra of a series of P2F-co-P3F copolymer synthesized by RAFT polymerization with different initial feed ratios of M2F and M3F.

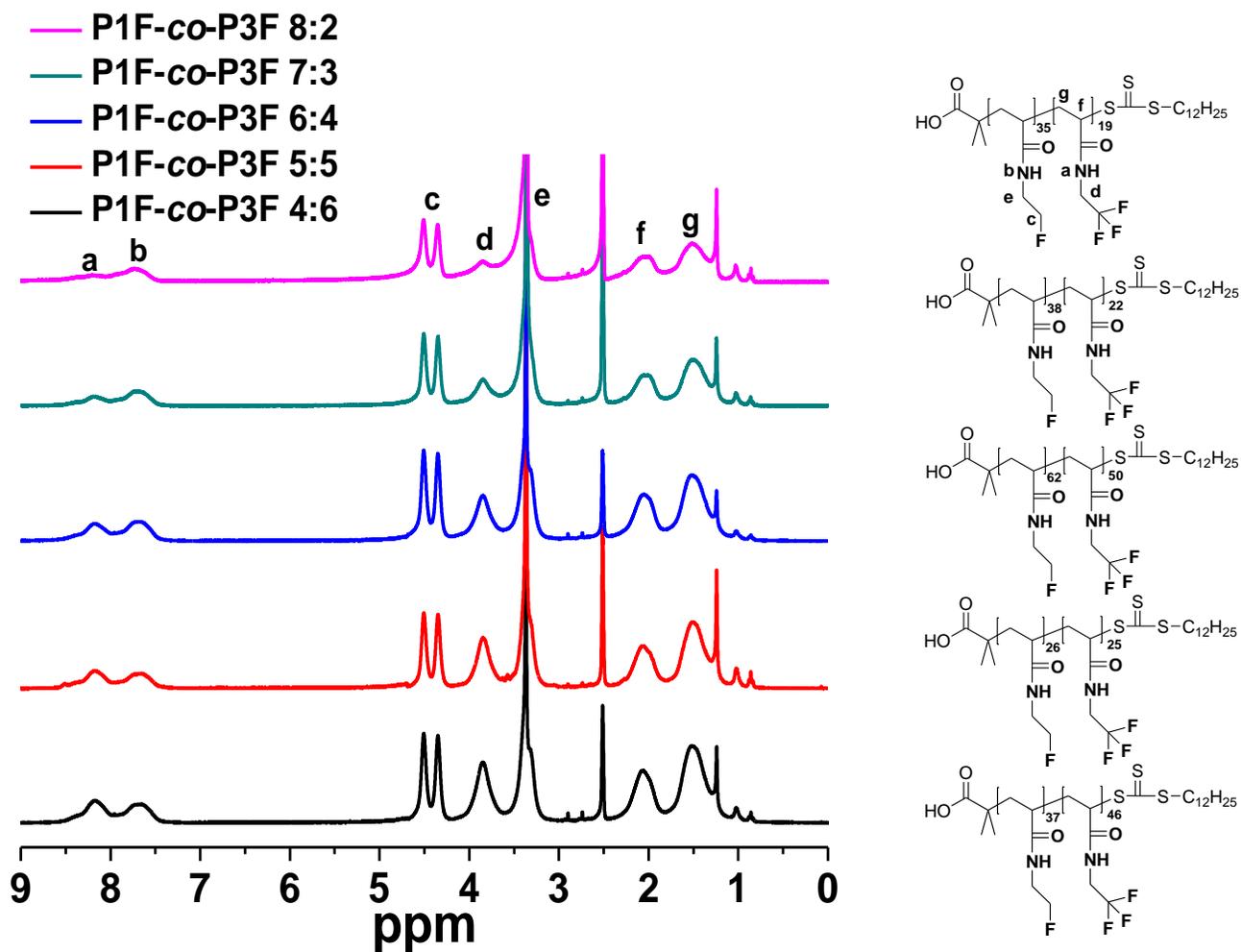


Figure S5. ^1H NMR spectra of a series of P1F-co-P3F copolymer synthesized by RAFT polymerization with different initial feed ratios of M1F and M3F.

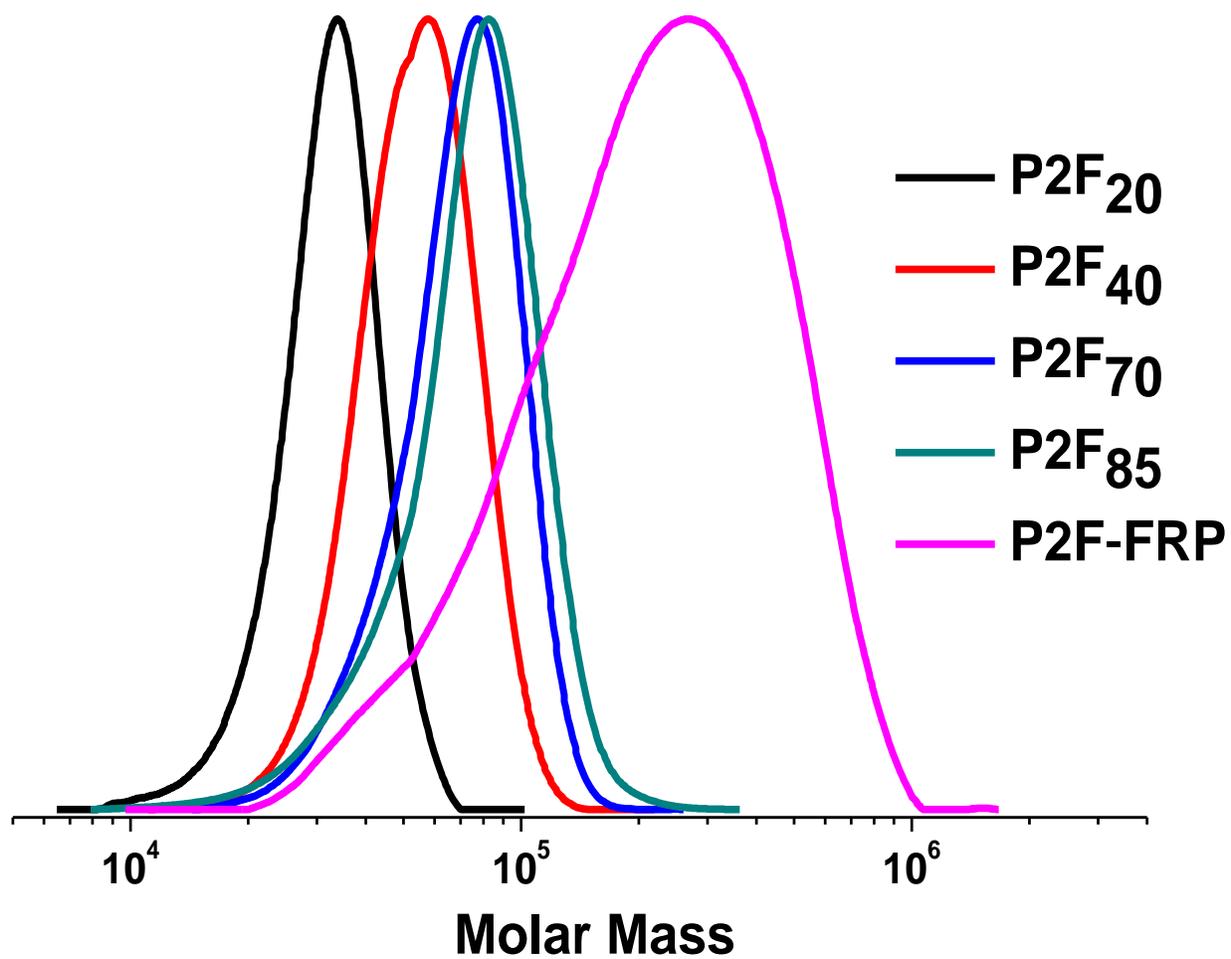


Figure S6. DMF GPC traces of a series of P2F homopolymer with different DP. P2F-FRP was synthesized by free radical polymerization.

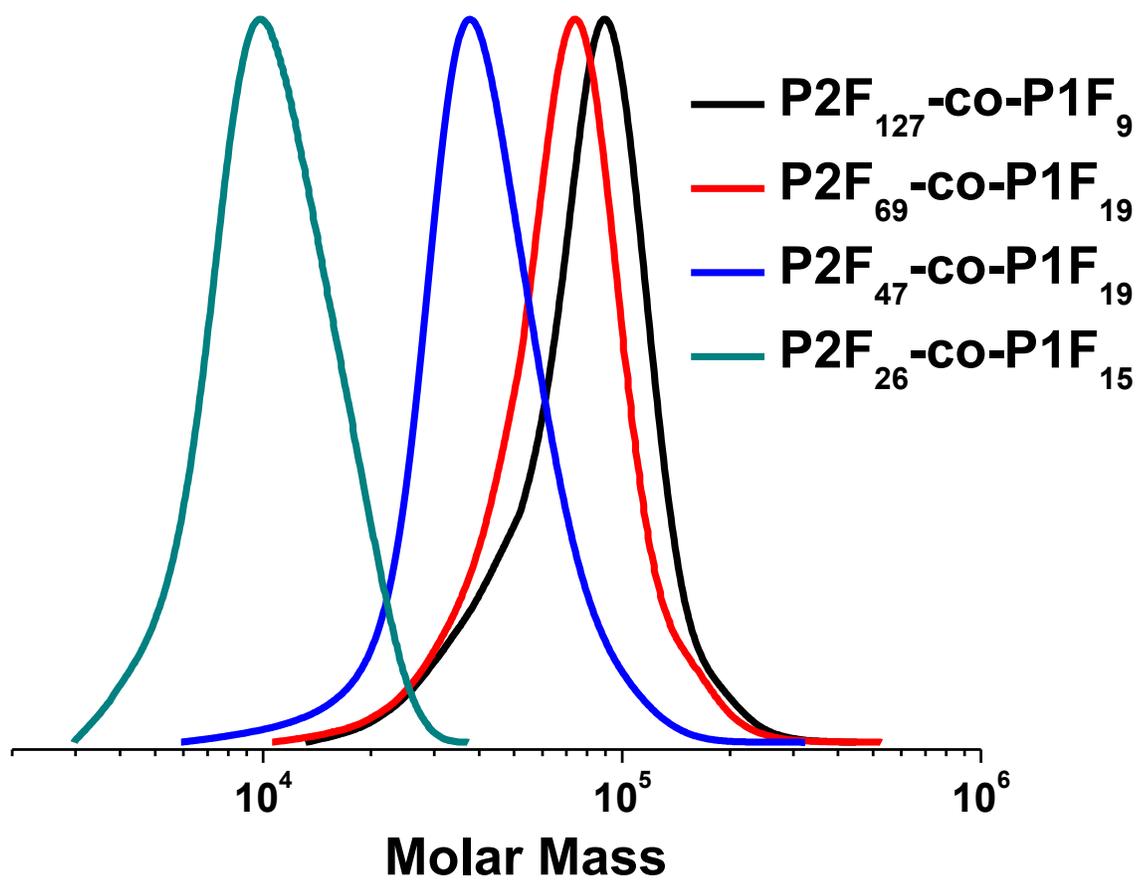


Figure S7. DMF GPC traces of a series of P2F-*co*-P1F copolymer.

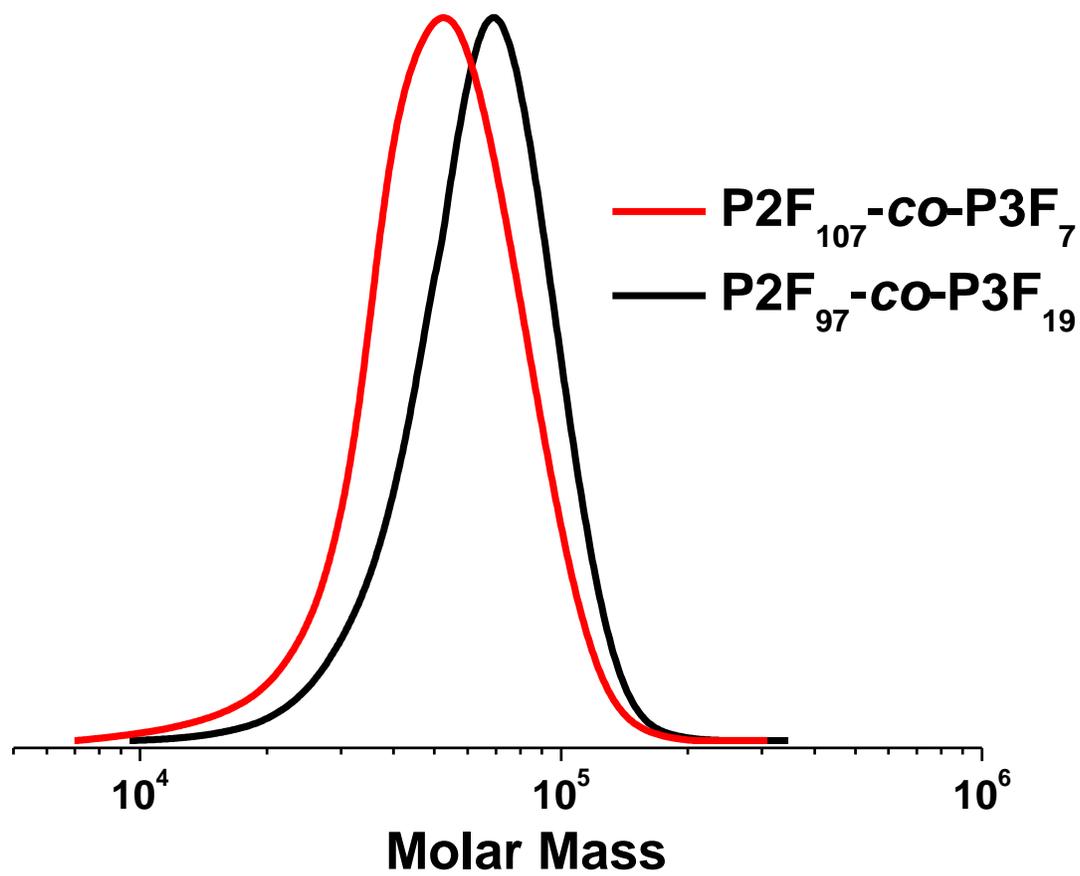


Figure S8. DMF GPC traces of a series of P2F-*co*-P3F copolymer.

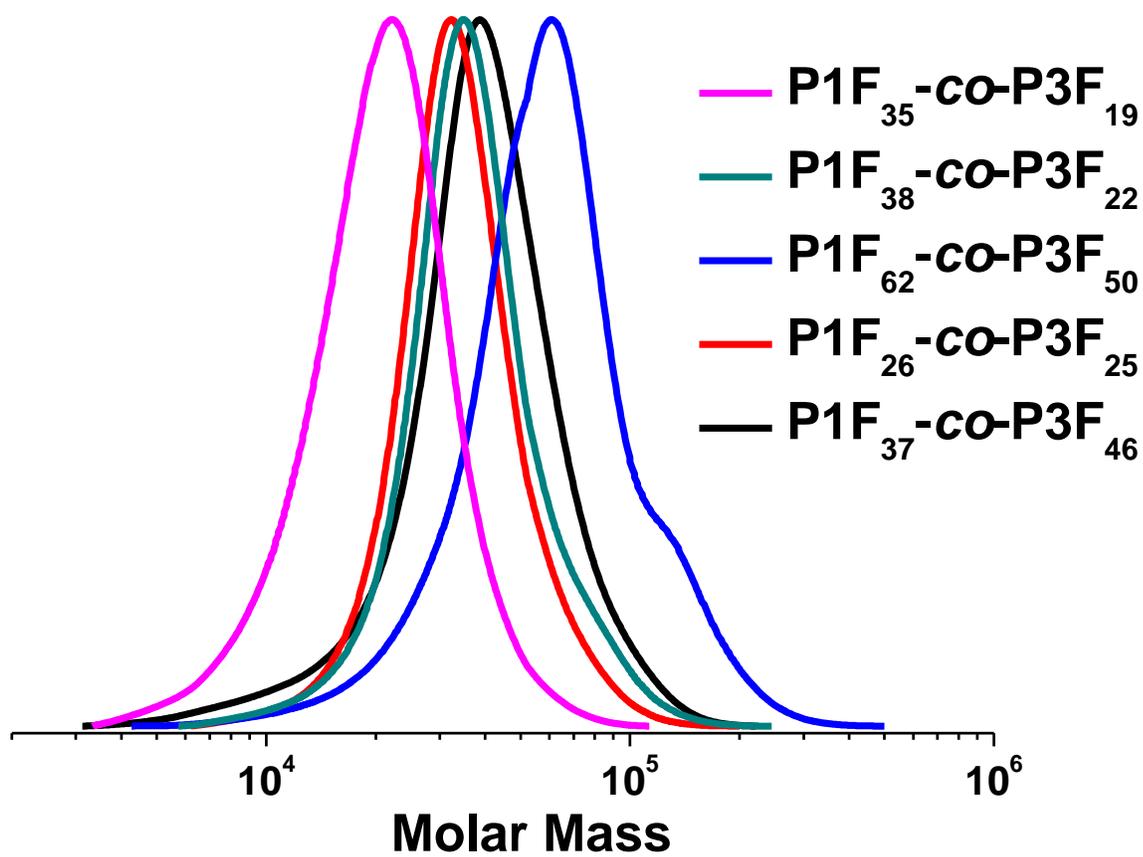


Figure S9. DMF GPC traces of a series of P1F-*co*-P3F copolymer.

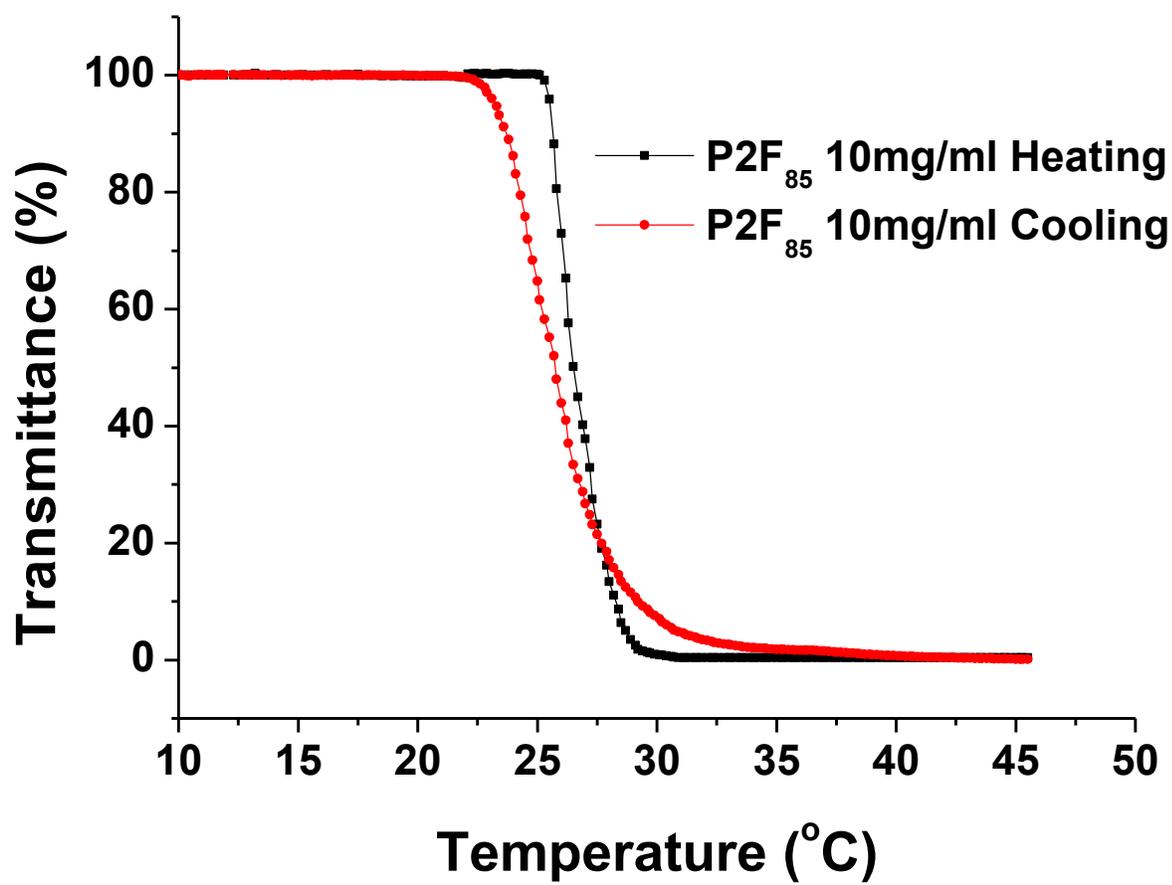


Figure S10. Thermoresponsiveness of 10 mg/mL aqueous solutions of P2F homopolymer with a heating and a cooling cycle.

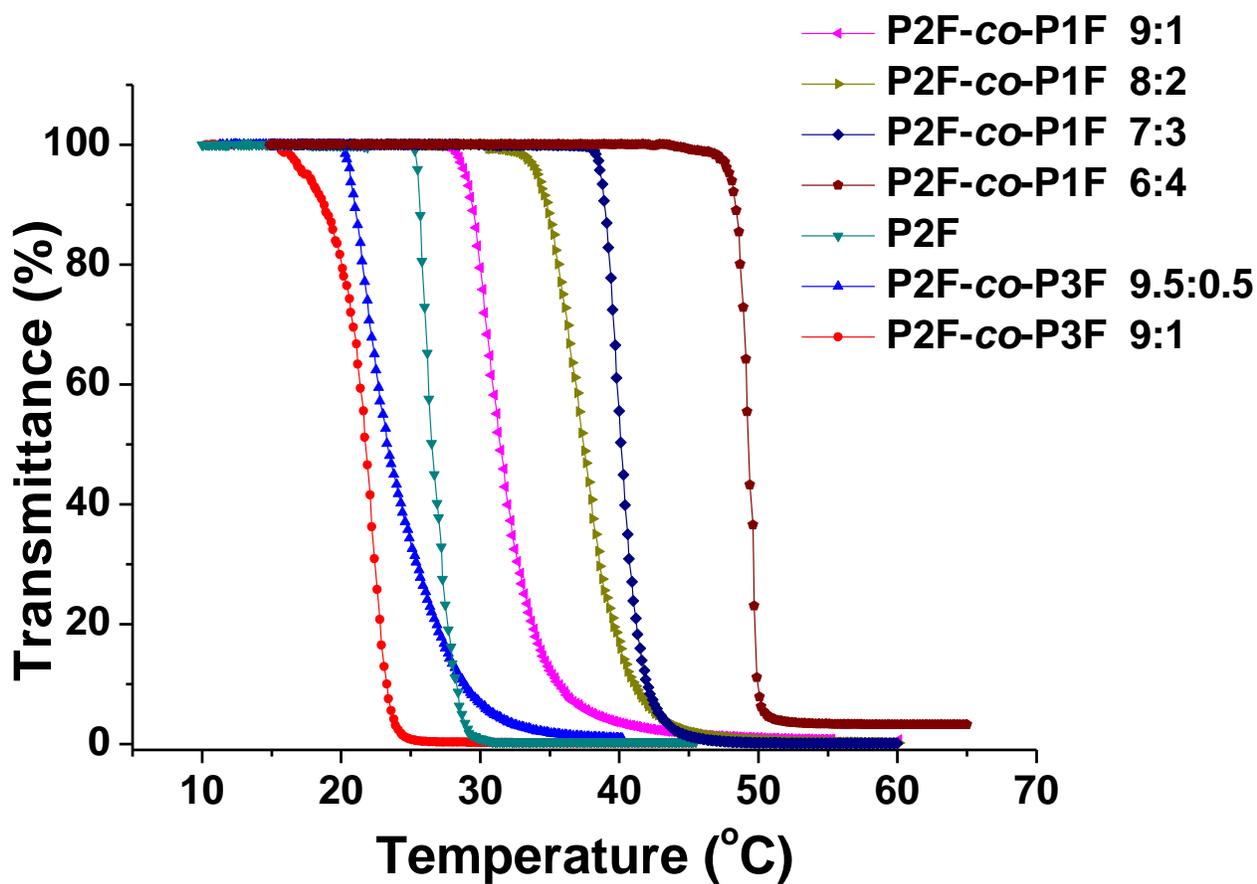


Figure S11. Thermoresponsiveness of 10 mg/mL aqueous solutions of P2F homopolymer, a series of P2F-co-P1F, and P2F-co-P3F copolymer measured by percent transmission at 650 nm.

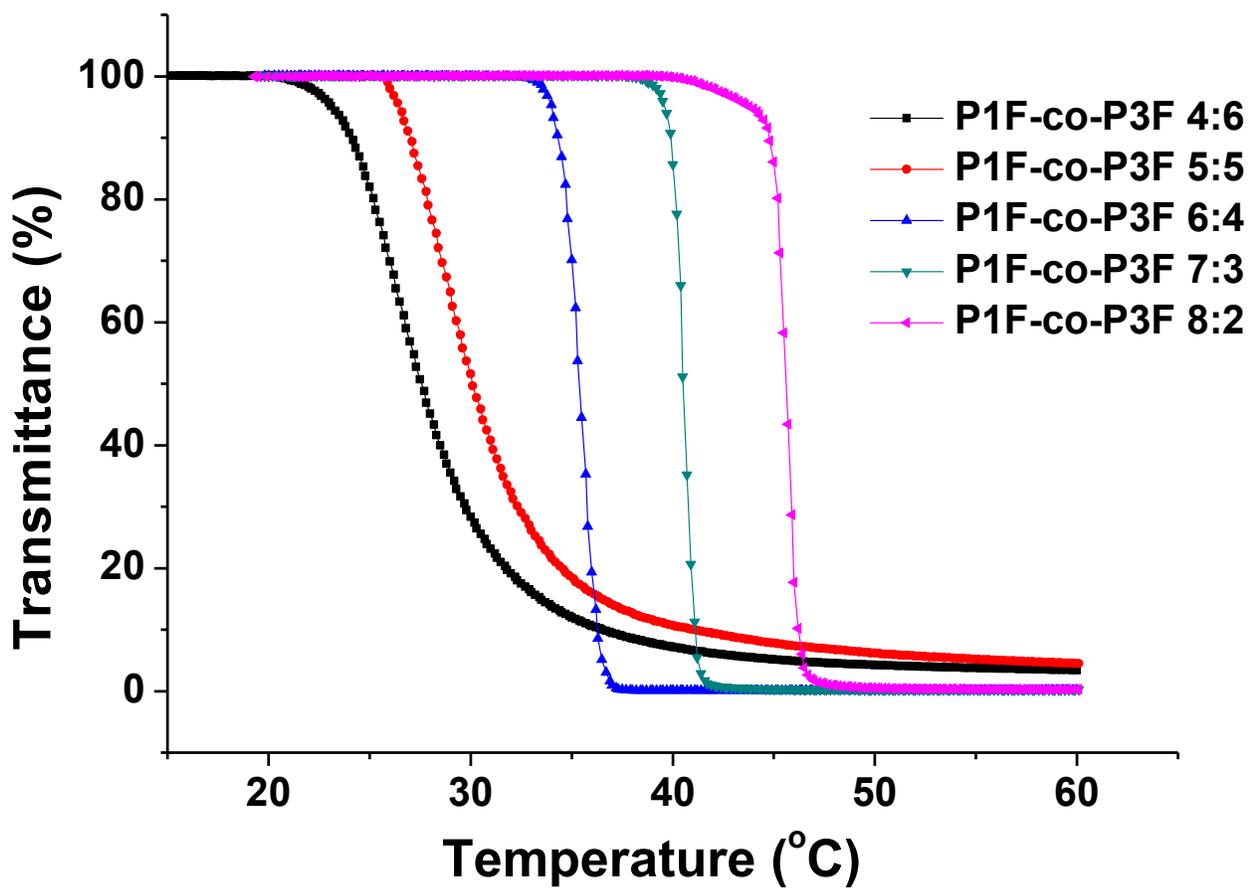


Figure S12. Thermoresponsiveness of 10 mg/mL aqueous solutions of P1F-*co*-P3F copolymer measured by percent transmission at 650 nm.