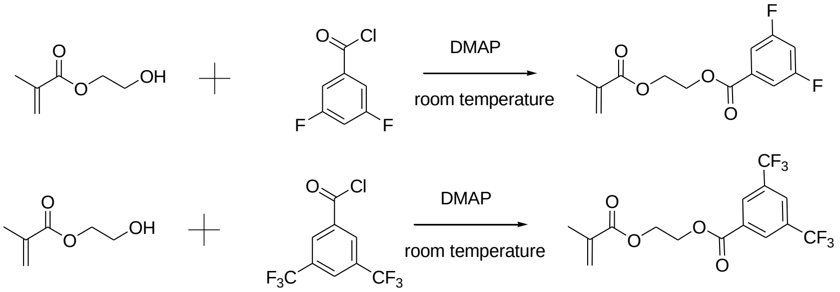
Table of Contents

Materials

2-(Pentamethyleneimino) ethanol (C6A), 2,2,2-trifluoroethyl methacrylate (TFE), 3,5-difluorobenzoyl chloride (DFBC), 3,5-bis(trifluoromethyl)benzoyl chloride (BTFBC), and 2-hydroxyethyl methacrylate (HEMA) were purchased from Sigma-Aldrich. 2-(Dibutylamino) ethanol (DBA) was purchased from TCI America Inc. Monomer 2-(diisopropyl amino) ethyl methacrylate (DPA-MA) was purchased from Polyscience company. PEG macroinitiator, MeO-PEG$_{114}$-Br, was prepared from 2-bromo-2-methyl propanoyl bromide and MeO-PEG$_{114}$-OH, according to the procedure in literature.[1] Other solvents and reagents were used as received from Sigma-Aldrich or Fisher Scientific, Inc.

Syntheses of ^{19}F monomers, 2-(methacryloyloxy) ethyl 3,5-bis(trifluoromethyl)benzoate (BTFB-MA) and 2-(methacryloyloxy) ethyl 3,5-difluorobenzoate (DFB-MA). The two ^{19}F monomers were synthesized using a similar method (Scheme S1). Synthesis of DFB-MA is described as a representative procedure. First, HEMA, 4-dimethylaminopyridine (DMAP) were dissolved in 50 mL dichloromethane. DFBC diluted with 20 mL dichloromethane was then added dropwise into a three-neck flask and stirred at room temperature overnight. The precipitated salt was removed by filtration. The residue was concentrated by rotary evaporation, and then passed through a column with a mixture of ethyl acetate and hexane (15:85). White solid products were purified by recrystallization in ethyl acetate and hexane (15:85).



Scheme S1. Syntheses of two ^{19}F monomers with different ^{19}F reporter moieties.

After syntheses, the monomers were characterized by ^{1}H (using tetramethylsilane (TMS) as the internal reference) or ^{13}C NMR in $CDCl_3$ on a Varian 500MHz ^{1}H NMR spectrometer. Matrix-assisted laser-desorption ionization time-of-light mass spectrometry (MALDI-TOF MS; Applied Biosystems, Voyager DE Pro) was performed in a positive-ion mode with a source temperature of 200 ℃ at a sample concentration of about 100 μM using alpha-cyano-4-hydroxycinnamic acid as the matrix. The characterization and yield for these monomers are as following:

2-(Methacryloyloxy) ethyl 3,5-difluorobenzoate (DFB-MA):

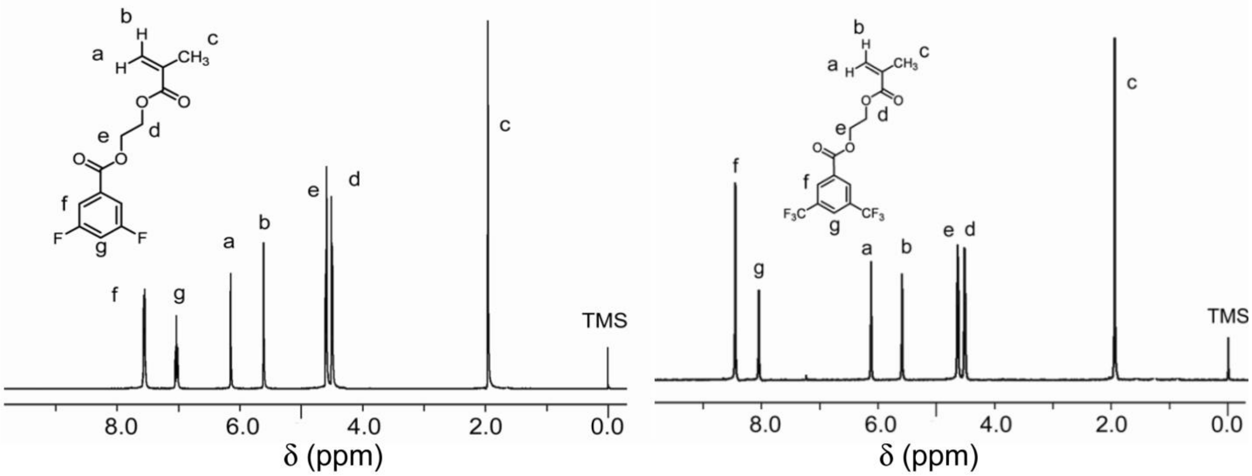
^{1}H NMR (TMS, $CDCl_3$, ppm): 7.65(m, 1H, -C*F*CHCF-), 7.08(m, 2H, -C*FCC*-), 6.09 (m, 1H, C*H*H=C(CH_3)-), 5.54(m, 1H, CH*H*=C(CH_3)-), , 4.61 (t, J = 6.2 Hz, 2H, -COOCH$_2$C*H$_2$*OCO-), 4.53 (t, 2H, -COOCH$_2$C*H$_2$*OCO-), 1.93 (s, 3H, CH2=C(C*H*3)-). Yield: 78 %.

^{13}C NMR (CDCl3, δ ppm): 166.23, 163.05, 135.81, 132.03, 131.89, 131.62, 129.32, 129.29, 125.99, 124.96, 123.71, 121.54, 63.48, 61.57, 17.19. Calculated M$_w$: 370.0640. Detected M$_w$: 371.2395 $(M + H)^+$.

2-(Methacryloyloxy) ethyl 3,5-bis(trifluoromethyl)benzoate (BTFB-MA):

^{1}H NMR (TMS, CDCl$_3$, ppm): 8.54(m, 1H, -C*F$_3$*CHCF$_3$-), 8.03(m, 2H, -C*F$_3$CC*-), 6.09 (m, 1H, C*H*H=C(CH$_3$)-), 5.54(m, 1H, CH*H*=C(CH$_3$)-), , 4.61 (t, J = 6.2 Hz, 2H, -COOCH$_2$C*H$_2$*OCO-), 4.53 (t, 2H, -COOCH$_2$C*H$_2$*OCO-), 1.93 (s, 3H, CH$_2$=C(C*H$_3$*)-). Yield: 76 %.

^{13}C NMR (CDCl3, δ ppm): 166.16, 162.00, 133.81, 132.03, 131.86, 131.59, 129.27, 125.92, 113.70, 111.53, 63.47, 61.54, 17.12. Calculated M$_w$: 270.0704. Detected M$_w$: 270.2760.



Scheme S2. ^{1}H NMR spectra of the above two ^{19}F monomers.

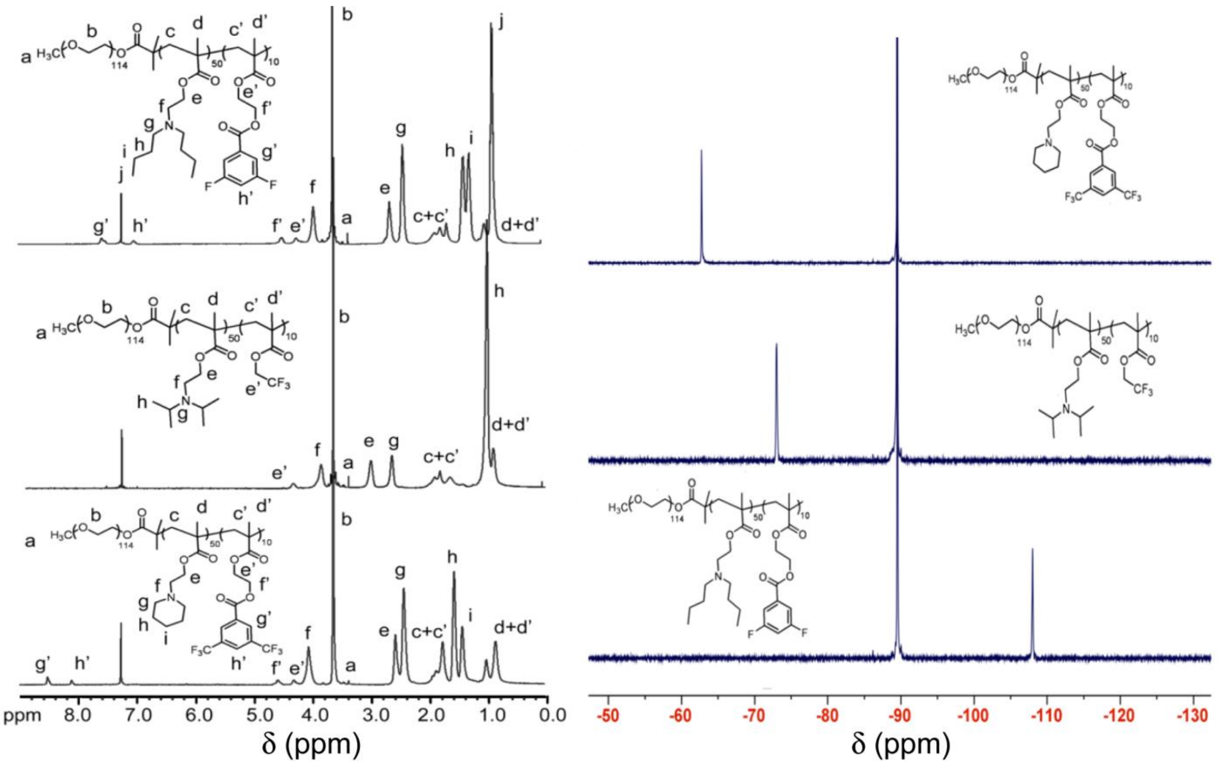
Syntheses of diblock copolymers PEO-*b*-P(R-*r*-F). Diblock copolymers PEO-*b*-P(R-*r*-F) with different tertiary amine (R) and fluorine (F) segments were synthesized by the atom transfer radical polymerization (ATRP) method. PEO-*b*-P(DPA$_{48}$-*r*-TFE$_{12}$) is described as an example to illustrate the procedure. First, DPA (1.0 g, 5 mmol), TFE (0.17 g, 1 mmol), N,N,N',N",N"-pentamethyldiethylenetriamine (PMDETA) (21 μL, 0.1 mmol), and MeO-PEG$_{114}$-Br (0.5 g, 0.1 mmol) were charged into a polymerization tube. Then a mixture of 2-propanol (1.5 mL) and DMF (1.5 mL) was added to dissolve the monomer and initiator. After three cycles of freeze-pump-thaw to remove oxygen, CuBr (14 mg, 0.1 mmol) was added into the reaction tube under nitrogen atmosphere, and the tube was sealed *in vacuo*. The polymerization was carried out at 40 ^{o}C for 8 hrs. After polymerization, the reaction mixture was diluted with THF, and passed through an Al$_2$O$_3$ column with tetrahydrofuran (THF) as solvent to remove the catalyst. The remaining THF solvent was removed by rotary evaporation. The residue was dialyzed in distilled water and lyophilized to obtain a white powder.

After syntheses, the polymers were dissolved in CDCl$_3$ and characterized by ^{1}H NMR and ^{19}F NMR, ^{1}H NMR spectra were obtained using tetramethylsilane (TMS) as the internal reference on a Varian 500MHz ^{1}H NMR spectrometer. ^{19}F spectra were obtained on a Varian ^{19}F 375MHz spectrometer by using perfluoro-15-crown-5-ether (PFCE) as the internal reference. The characterizations for polymers are as following:

Poly(ethylene oxide)-*b*-poly[2-(dibutylamino) methacrylate-*r*-2-(methacryloyloxy) ethyl 3,5-difluorobenzoate] (PEO-*b*-P(DBA-*r*-DFB)): ^{1}H NMR (TMS, CDCl$_3$, ppm): 7.52(-CFC*H*C-), 6.95(-CF*CH*CF-), 4.42 (-COOCH$_2$C*H$_2$*OCO-), 4.17(-COOCH$_2$C*H$_2$*OCO-), 3.88(-NC*H$_2$*CH$_2$OCO-), 3.56(-OCH$_2$CH$_2$-), 3.29 (C*H$_3$*OCH$_2$CH$_2$-), 2.58 ($-NCH_2C\mathit{H_2}OCO-$), 2.36($-N(C\mathit{H_2}CH_2CH_2CH_3)_2$), 1.71~1.60($-C\mathit{H}CH_3-$), 1.32($-N(CH_2C\mathit{H_2}CH_2CH_3)_2$, 1.22 ($-(-N(CH_2CH_2C\mathit{H_2}CH_3)_2$, 0.96 ($-CHC\mathit{H_3}-$), 0.82($-N(CH_2CH_2CH_2C\mathit{H_3})_2$). **^{19}F NMR** (PFCE, $CDCl_3$, ppm): -60.7($-C\mathit{F}CHC\mathit{F}-$).

Poly-(ethylene oxide)-*b*-poly[2-(diisopropylamino) ethyl methacrylate-*r*-trifluoroethyl methacrylate] (PEO-*b*-P(DPA-*r*-TFE)): ^{1}H NMR (TMS, $CDCl_3$, ppm): 4.25($-CF_3C\mathit{H_2}O-$), 3.77($-NC\mathit{H_2}CH_2OCO-$), 3.57($-OCH_2CH_2-$), 3.31 ($C\mathit{H_3}OCH_2CH_2$), 2.92($-NCH_2C\mathit{H_2}OCO-$), 2.56($-NC\mathit{H}(CH_3)_2$), 1.84~1.57($-C\mathit{H}CH_3-$), 0.94 ($-NCH(C\mathit{H_3})_2$), 0.83($-CHCH_3-$). **^{19}F NMR** (PFCE, $CDCl_3$, ppm): -73.5($C\mathit{F_3}CH_2O-$).

Poly(ethylene oxide)-*b*-poly[2-(pentamethylene imino) methacrylate-*r*-2-(methacryloyloxy) ethyl 3,5-bis(trifluoromethyl) benzoate] (PEO-*b*-P(C6A-*r*-BTFB)): ^{1}H NMR (TMS, $CDCl_3$, ppm): 8.54($-CF_3C\mathit{H}C-$), 8.13($-CF_3C\mathit{H}CF_3-$), 4.60($-COOCH_2C\mathit{H_2}OCO-$), 4.31($-COOCH_2C\mathit{H_2}OCO-$), 4.08($-NC\mathit{H_2}CH_2OCO-$), 3.66($-OCH_2CH_2-$), 3.29($C\mathit{H_3}OCH_2CH_2-$), 2.60($-NCH_2C\mathit{H_2}OCO-$), 2.46($-N(C\mathit{H_2}CH_2CH_2CH_2C\mathit{H_2})$), 1.91~1.80($-C\mathit{H}CH_3-$), 1.60($N(CH_2C\mathit{H_2}CH_2C\mathit{H_2}CH_2)$),, 1.46 ($N(C\mathit{H_2}CH_2C\mathit{H_2}CH_2C\mathit{H_2})$), 0.96 ($-CHC\mathit{H_3}-$). **^{19}F NMR** (PFCE, $CDCl_3$, ppm): -108.5($-C\mathit{F_3}CHC\mathit{F_3}-$).



Scheme S3. ^{1}H (500 MHz) and ^{19}F (375 MHz) NMR spectra of the three copolymers in $CDCl_3$.

GPC measurement. GPC (gel permeation chromatography) measurements were carried out on Viscotek GPCmax with triple-detector array (TDA302). Two PLgel 5 μm MIXED-D columns by Polymer Labs were placed in a thermostat at 35 °C, and THF was used as eluent with a flow rate of 1 mL/min. A series of narrowly dispersed polystyrenes were used as the standards, and software Omnisec 4.1 was applied to calculate the molecular weight and polydispersity index (PDI).

Preparation of micelle nanoparticles. Micelles were prepared following a solvent evaporation method as previously published.[2] Briefly, in the example of PEO-*b*-P(DPA-*r*-TFE), 24 mg of the copolymer was first dissolved in 1 mL THF and then added into 4 mL distilled water dropwise under sonication. The THF was allowed to evaporate for 4 hrs by air stream. Then distilled water was added to adjust the polymer concentration to 4 mg/mL as a stock solution. After micelle formation, the nanoparticles were characterized by transmission electron microscopy (TEM, JEOL 1200 EX model) to examine micelle size and morphology, dynamic light scattering (DLS, Malvern MicroV model, He-Ne laser, λ= 632 nm) for hydrodynamic diameter (D_h).

^{19}F NMR spectroscopy study of PEO-*b*-P(DPA-*r*-TFE) nanoprobes. First, for the measurement of T_2 of PEO-*b*-P(DPA-*r*-TFE) copolymers with different TFE compositions, micelle solution was prepared at a copolymer concentration of 5 mg/mL in deuteroxide. Then 0.25 mL of copolymer solution was mixed with 0.25 mL pH 4.0 acetate buffers (100 mM). After vortex, the samples were analyzed on a Varian ^{19}F 375 MHz (9.4T) vertical wide-bore NMR system. Trifluoroacetic acid (TFA), as an external reference, was sealed in a micro-tube which was inserted into each NMR sample tube so that the sample and the reference were concentric. T_2 of each sample was analyzed using a Carr Purcell Meiboom Gill (CPMG) sequence with a repetition of 2.5 seconds and 13 τ values. T_2 values were obtained by fitting the ^{19}F signal integral intensities vs τ to a single exponential decay using SigmaPlot 9.01 (Systat software, San Jose, CA). ^{19}F signal intensity was also measured as a function of copolymer composition. The results were shown in Figure S2d-e. For pH titration studies, the PEO-*b*-P(DPA_{48}-*r*-TFE_{12}) micelle solution was first prepared at 5 mg/mL in deuteroxide. Then the pH of the copolymer solution was adjusted by adding deuterated acetic acid (acidify) or NaOH (basify) to achieve the designated values as shown in Figure 1a. ^{19}F signal intensity was then plotted as a function of pH to yield Figure 1b.

^{19}F-MR imaging studies. The images in Fig. 2 were acquired using a 9.4T Agilent (Varian) horizontal bored scanner and a surface coil of 2cm diameter as both excitation and receiving RF device. A standard gradient echo sequence (GEMS) was used to record the ^{19}F MRI images with repetition time TR=100 ms, echo time TE=1.9 ms, flip angle FA=38.5, matrix size 64x64, field of view FOV=60x60 mm, slice thickness=5mm and NT=512. The SNR ratios calculated from phantom images were estimated as (average signal intensity in region of interest)/(standard deviation of signal intensity of background). For multi-chromatic ^{19}F MR imaging studies, a steady-state free procession (SSFP) pulse sequence was used with a proper sinc shaped excitation pulse width and the resonance frequencies, which were set right on the chemical shifts of ^{19}F reporters.[3] ^{19}F resonance was manually set onto a specific peak to selectively acquire three ^{19}F reporter images sequentially. Several key parameters are as follows: TR = 5ms, TE = 1.54 ms, FA = 50, NT = 2048, matrix size = 64x64, FOV = 40x40mm, and slice thickness = 8mm. A typical acquisition time was 36 mins.

References

1. Bronstein, L. M.; Sidorov, S. N.; Zhirov, V.; Zhirov, D.; Kabachii, Y. A.; Kochev, S. Y.; Valetsky, P. M.; Stein, B.; Kiseleva, O. I.; Polyakov, S. N.; Shtykova, E. V.; Nikulina, E. V.; Svergun, D. I.; Khokhlov, A. R. *J. Phys. Chem. B* **2005**, *109*, 8786–18798.
2. Nasongkla, N.; Bey, E.; Ren, J. M.; Ai, H.; Khemtong, C.; Guthi, J. S.; Chin, S. F.; Sherry, A. D.; Boothman, D. A.; Gao, J. M., Multifunctional polymeric micelles as cancer-targeted, MRI-ultrasensitive drug delivery systems. *Nano. Lett.* **2006**, *6*, 2427-2430.
3. Bernstein, M.A., King, K.F., Zhou X.J., Handbook of MRI Pulse Sequences, Elsevier Academic Press, **2004**, Pages 39-43.

Table S1. Syntheses and characterization of PEO-*b*-P(DPA-*r*-TFE) diblock copolymers.

Copolymer	Yield (%)	MONOMER[a]		MONOMER[b]		TFE mol %	$M_{n,GPC}$ (×10^{-4} D)[c]	PDI[b]	$M_{n,1H\ NMR}$ (×10^{-4} D)[d]
		DPA	TFE	DPA	TFE				
PEO-*b*-P(DPA_{53}-*r*-TFE_3) (P1)	71	57	3	53	3	5.3	1.72	1.52	1.68
PEO-*b*-P(DPA_{52}-*r*-TFE_8) (P2)	62	55	5	52	7	11.8	1.81	1.47	1.72
PEO-*b*-P(DPA_{48}-*r*-TFE_{12}) (P3)	71	50	10	48	12	20.0	1.87	1.56	1.72
PEO-*b*-P(DPA_{37}-*r*-TFE_{21}) (P4)	81	40	20	37	21	36.2	1.72	1.38	1.64
PEO-*b*-P(DPA_{23}-*r*-TFE_{31}) (P5)	73	30	30	23	31	57.4	1.62	1.61	1.51
PEO-*b*-P(DPA_{16}-*r*-TFE_{44}) (P6)	65	20	40	16	44	73.3	1.68	1.49	1.58

[a] DPA and TFE molar ratio used for reaction. [b] ^{1}H NMR characterization of DPA and TFE ratios; [c] Number-averaged molecular weight (M_n) and polydispersity index (PDI=M_w/M_n) were determined by GPC using THF as the eluent; [d] Determined by ^{1}H NMR.

Table S2. pKa and ^{19}F NMR parameters of PEO-*b*-P(DPA-*r*-TFE) nanoprobes.

Polymer	pK_a	Chemical shift (ppm)	Peak width (ppm)	T_2 (ms)
P1	6.3	3.23	73.13	47.4
P2	6.2	3.16	128.5	49.6
P3	6.1	3.05	147.7	43.5
P4	5.1	2.83	180.6	17.6
P5	4.2	2.64	377.3	4.7
P6	--	--	--	--

Table S3. Characterization of PEO-*b*-P(R-*r*-F) diblock copolymers.

Co-Polymer	pK_a	Yield (%)	Monomer[a]		Monomer[b]		M_n,GPC (×10^4 D)[c]	PDI
			R	F	R	F		
PEO-*b*-P(C6A-*r*-BTFB)	7.0	83	60	10	62	9	1.67	1.34
PEO-*b*-P(DPA-*r*-TFE)	6.1	89	60	15	56	10	2.17	1.43
PEO-*b*-P(DBA-*r*-DFB)	5.0	87	60	7	55	7	2.08	1.29

[a] R and F molar ratio used for reaction; [b] ^{1}H NMR ratio of R and F; [c] Number-averaged molecular weight (M_n) and polydispersity index (PDI=M_w/M_n) were determined by GPC using THF as the eluent.

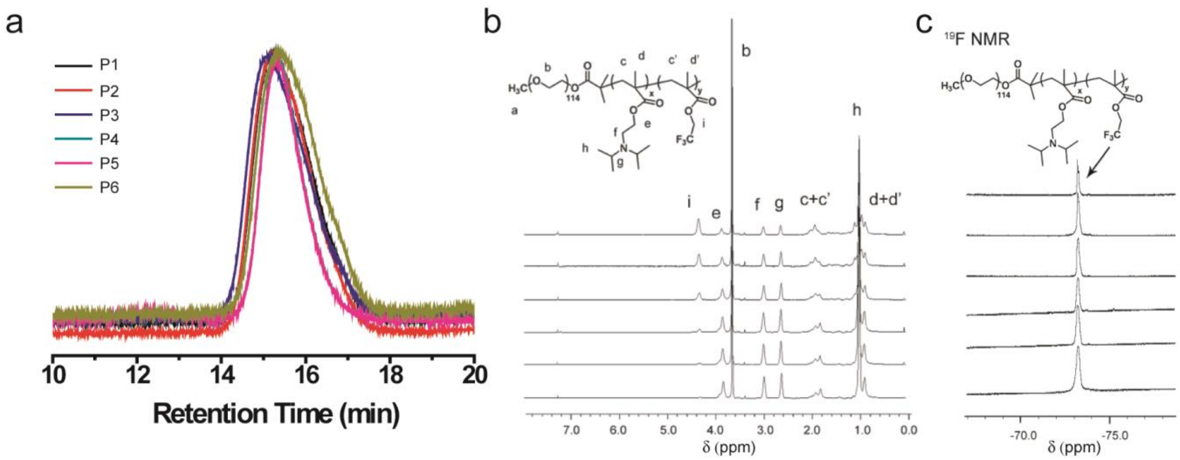


Figure S1. Characterization of PEO-*b*-P(DPA-*r*-TFE) copolymers with increasing molar fractions of TFE component: (a) GPC chromatograms in THF, (b) ^{1}H NMR and c) ^{19}F NMR spectra in $CDCl_3$. P1 to P6 (see Table S1 for detailed compositions) spectrum was arranged from bottom to top, respectively, in both b and c.

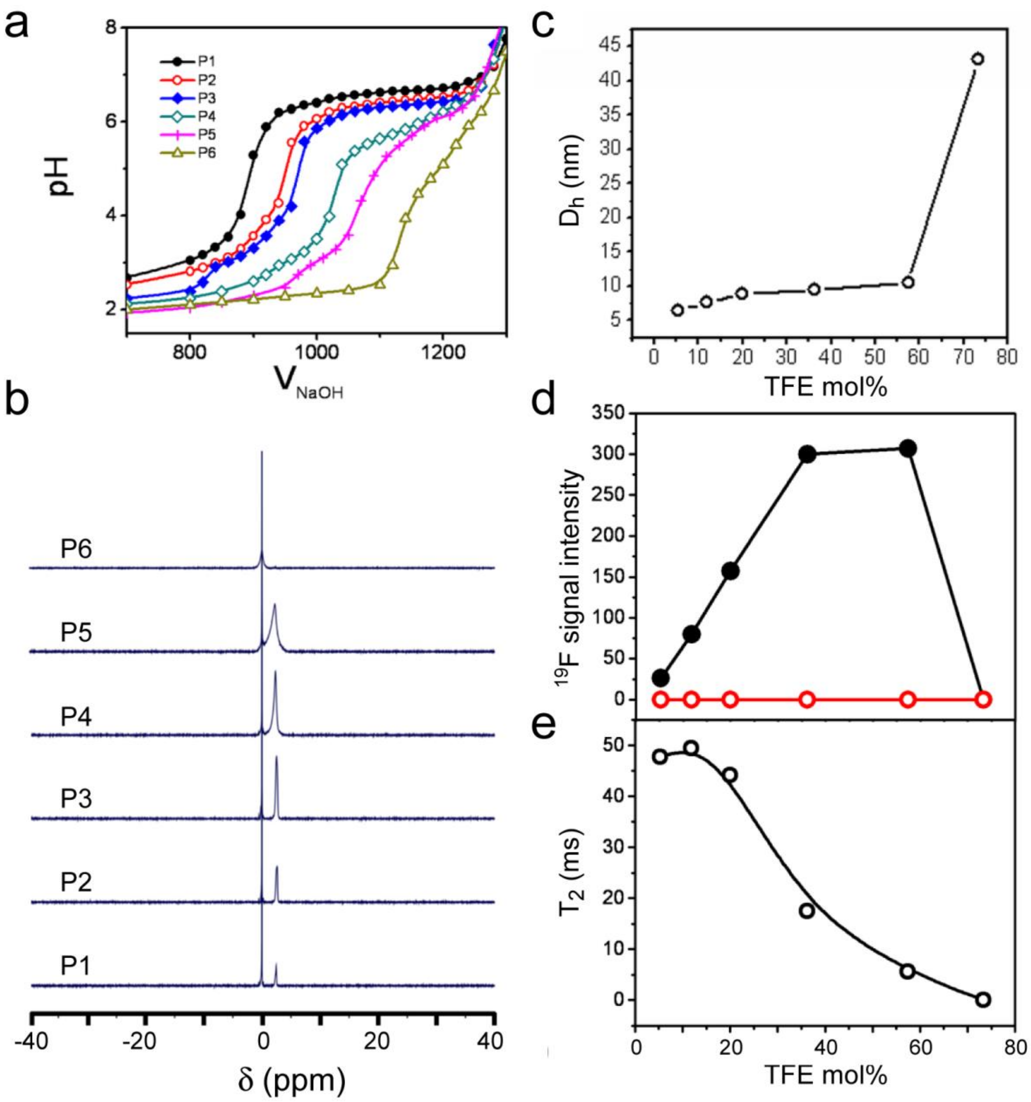


Figure S2. (a) pH titration curves of PEO-*b*-P(DPA-*r*-TFE) copolymers (1 mg/mL) with different molar fractions of TFE components. (b) ^{19}F NMR spectra and (c) hydrodynamic diameters (D_h in nm) of PEO-*b*-P(DPA-*r*-TFE) copolymers (P1-P6) in aqueous pH 4.0 acetate buffer solutions. TFA was added as an external reference. (d) ^{19}F signal intensity and (e) T_2 relaxation time (in ms) as a funciton of TFE content at pH 4.0.

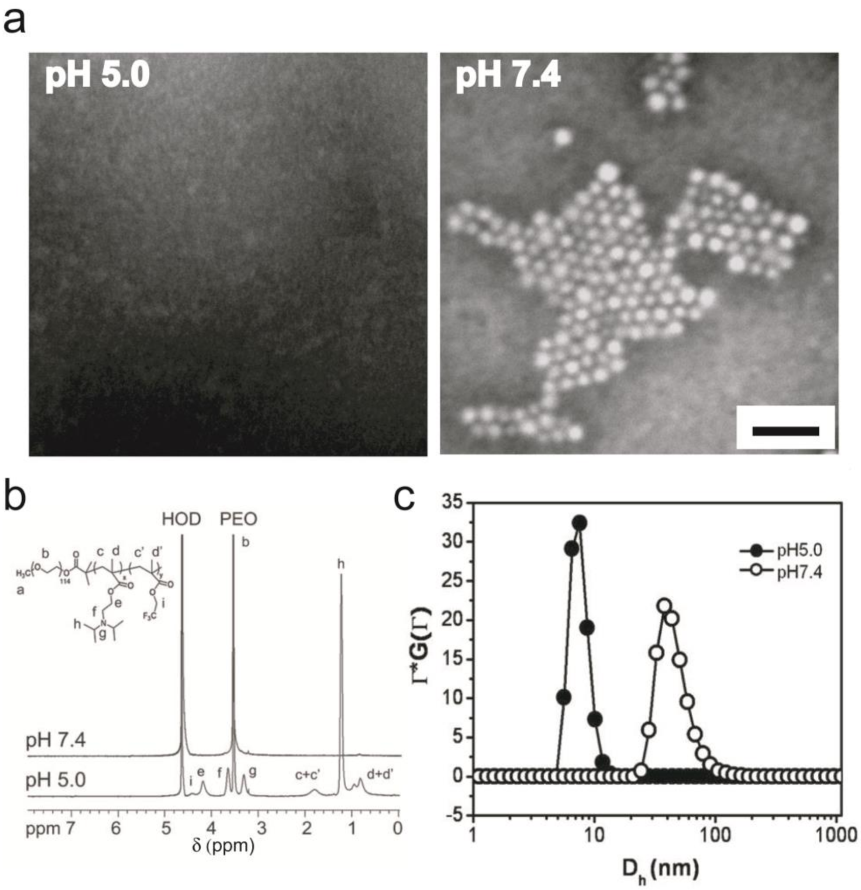


Figure S3. (a) TEM images, (b) ^{1}H NMR spectra (in D_2O), and (c) DLS histograms of PEO-*b*-P(DPA_{48}-*r*-TFE_{12}) nanoprobes as micelles in pH 7.4 solution and as unimers in pH 5.0 solution, respectively. Scale bar = 100 nm in both TEM images.

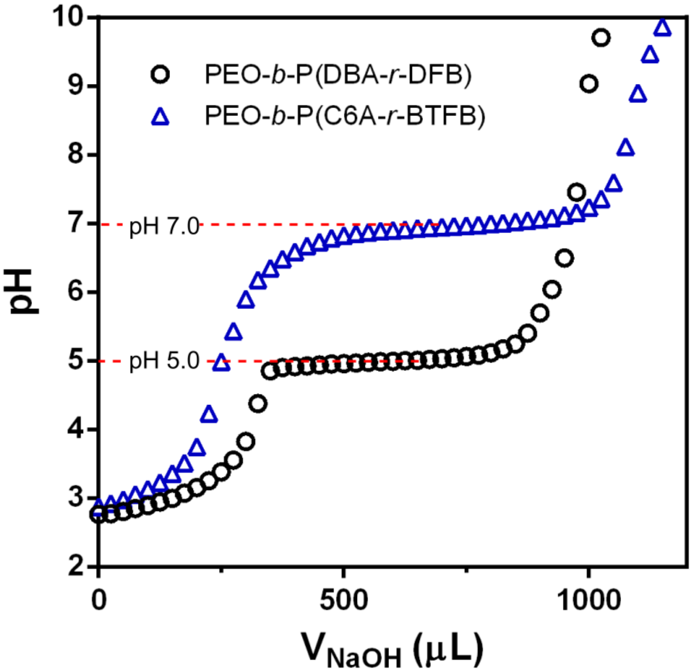


Figure S4. pH titration curves of PEO-*b*-P(DBA-*r*-DFB) and PEO-*b*-P(C6A-*r*-BTFB) (1mg/mL). The values of pK_a were measured at 5.0 and 7.0 for PEO-*b*-P(DBA-*r*-DFB) and PEO-*b*-P(C6A-*r*-BTFB), respectively.

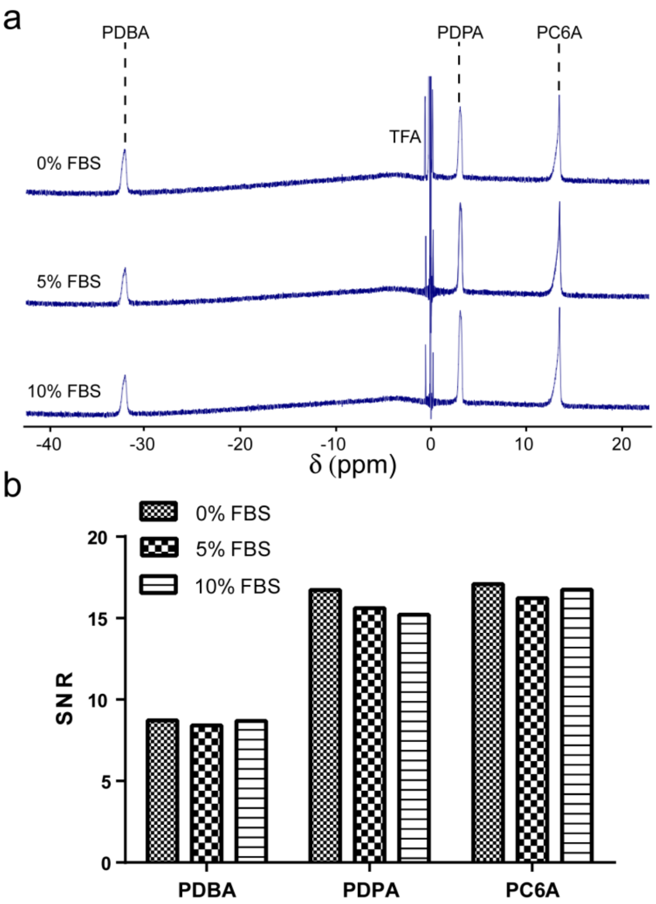


Figure S5. (a) ^{19}F spectra of a mixture of three PEO-*b*-P(R-*r*-F) nanoprobes in pH 4.5 buffer with addition of fetal bovine serum (FBS, 5 or 10%). TFA was used as an external reference. (b) Signal-to-noise ratio (SNR) of each ^{19}F reporter in pH 4.5 with addition of different amounts of FBS. All the NMR spectra were acquired under the same condition.