Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supplementary Information

Non-covalent interactions in controlling pH-responsive behaviors of self-assembled nanosystems

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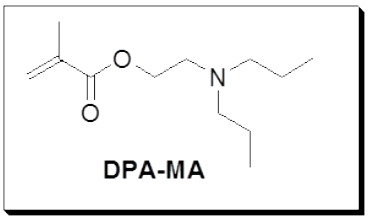
Materials

N-Hydroxysuccinimidal ester of tetramethyl rhodamine (NHS-TMR) was purchased from the Invitrogen Company. PEO macroinitiator, MeO-PEO_{114}-Br, was prepared from 2-bromo-2-methyl propanoyl bromide and MeO-PEO_{114}-OH according to the procedure in literature[1]. Monomers 2-(diisopropyl amino)ethyl methacrylate (iDPA-MA) were purchased from Polyscience company. Monomers such as 2-(dipropylamino) ethyl methacrylate (nDPA-MA) monomer were synthesized following a previous publication[1]. Bromopropane, bromobutane, bromopentane, ethanolamine, sodium salts and methacryloyl chloride were purchased from Sigma-Aldrich. Other solvents and reagents were used as received from Sigma-Aldrich or Fisher Scientific Inc.

Syntheses of methacrylate monomers

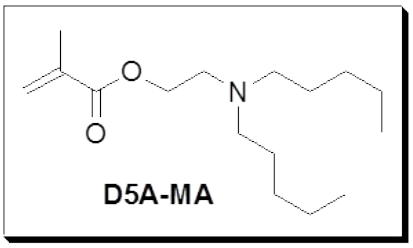
Methacrylate monomers were synthesized following a published method[2]. Synthesis of 2-(dipropylamino) ethyl methacrylate (DPA-MA) is described here as an example. First, ethanolamine (12.2g, 0.2 mol) and bromopropane (49.2 g, 0.4 mol) were dissolved in 400 mL acetonitrile, and Na_2CO_3 (53.0 g, 0.5 mol) was added to the solution. After overnight reaction, the solution was filtered to remove the precipitated NaBr salt and extra Na_2CO_3. CH_3CN solvent was removed by rotovap. The resulting residue was distilled in vacuo (40~45 °C at 0.05 mm Hg) as a colorless liquid to obtain 2-(dipropylamino) ethanol. Then 2-(dipropylamino) ethanol (21.3g, 0.1 mol), triethylamine (10.1 g, 0.1 mol), and inhibitor hydroquinone (0.11g, 0.001mol) were dissolved in 100 mL CH_2Cl_2 and methacryloyl chloride (10.4g, 0.1 mol) was added dropwise into a three-neck flask. The solution was refluxed overnight. After reaction, the solution was filtered to remove the precipitated triethylamine-HCl salts, and CH_2Cl_2 solvent was removed by rotovap. The resulting residue was distilled in vacuo (47-53 °C at 0.05 mm Hg) as a colorless liquid.

Characterization of some synthesized monomers



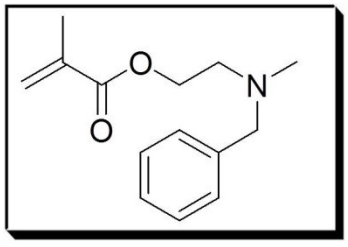
2-(Dipropylamino) ethyl methacrylate (DPA-MA)

^{1}H NMR (TMS, $CDCl_3$, ppm): 6.10 (br, 1H, C*H*H=C(CH_3)-), 5.54 (br, 1H, CH*H*=C(CH_3)-), 4.07 (t, 2H, -OC$H_2$$CH_2$N-), 3.01 (t, 2H, -$OCH_2C$$H_2$N-), 2.68 (t, 4H, -N(C$H_2$$CH_2$$CH_3$)$_2$, 1.94 (s, 3H, CH_2=C(CH_3)-), 1.43 (m, 4H, -N($CH_2C$$H_2$$CH_2CH_3$)$_2$), 1.01(t, 6H, -N($CH_2CH_2C$$H_3$)$_2$)



2-(Dipentylamino) ethyl methacrylate (D5A-MA)

^{1}H NMR (TMS, $CDCl_3$, ppm): 6.10 (br, 1H, C*H*H=C(CH_3)-), 5.55 (br, 1H, CH*H*=C(CH_3)-), 4.20 (t, 2H, -OC$H_2$$CH_2$N-), 2.74 (t, 2H, -$OCH_2C$$H_2$N-), 2.45 (t, 4H, -N(C$H_2$$CH_2CH_2$ CH_2CH_3)$_2$, 1.94 (s, 3H, CH_2=C(CH_3)-), 1.43 (m, 4H, -N($CH_2C$$H_2CH_2$ CH_2CH_3)$_2$), 1.30 (m, 4H, -N($CH_2CH_2C$$H_2$ CH_2CH_3)$_2$), 1.24 (m, 4H, -N($CH_2CH_2CH_2$ CH_2CH_3)$_2$), 0.88 (t, 6H, -N($CH_2CH_2CH_2$ $CH_2C$$H_3$)$_2$),



2-(methylbenzylamino)ethyl methacrylate (MBA-MA).

^{1}H NMR (TMS, CDCl3, ppm): 7.37, 7.30, 7.26 (m, 5H, -$CH_2C_6$$H_5$), 6.12 (br, 1H, C*H*H=C(CH_3)-), 5.58 (br, 1H, CH*H*=C(CH_3)-), 4.29 (t, 2H, -OC$H_2$$CH_2$N-), 3.59 (s, 2H, -$OCH_2C$$H_2$N-), 2.72 (t, 2H, -N(C$H_2C_6H_5$)($CH_3$)), 2.32 (s, 3H, -N($CH_2C_6H_5$)(C$H_3$), 2.05 (m, 4H, (s, 3H, CH_2=C(CH_3)-))

Exemplary syntheses of PEG-*b*-PR block copolymers

PEG-b-PR copolymers were first synthesized by atom transfer radical polymerization (ATRP) method[2]. First, DPA-MA (1.7 g, 8 mmol), PMDETA (21 μL, 0.1 mmol), and MeO-PEO_{114}-Br (0.5 g, 0.1 mmol) were charged into a polymerization tube. Then a mixture of 2-propanol (2 mL) and DMF (2 mL) was added to dissolve the monomer and initiator. After three cycles of freeze-pump-thaw to remove oxygen, CuBr (14.4 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 °C for 12 hours. After polymerization, the reaction mixture was diluted with 10 mL THF, and passed through an Al2O3 column to remove the catalyst. The THF solvent was removed by rotovap. The residue was dialyzed in distilled water and lyophilized to obtain a white powder.

Preparation of micelle solution

For each copolymer, the stock solution of micelles was prepared following a solvent evaporation method as previously reported[3]. In the example of PEO-*b*-nPDPA micelle solution, 20 mg of the copolymer was first dissolved in 2.0 mL THF and then added into 10 mL deionized water dropwise under sonication. The THF was removed through ultrafiltration with (100 KD) membrane for five times. Then deionized water was added to adjust the polymer concentration to 5 mg/mL as a stock solution.

pH titration of copolymers

The stock solution of copolymers (5.0 mg/ml) was first diluted to a final polymer concentration of 2.0 mg/ml in the deionized water. NaCl was added to adjust the salt concentration to 150 mM. pH titration was carried out by adding small volumes (1 μL increment) of 4.0 M HCl solution under stirring to 20 mL micelle solution. The pH values were measured using a Mettler Toledo pH meter with a microelectrode. Without specific mention, pH titration of copolymers followed similar procedures with fixed weight concentration of polymers at 2.0 mg/ml in the presence of 150 mM NaCl.

For the pH titration of PEO_{114}-*b*-PDPAx block copolymers with different hydrophobic chain length as shown in Figure 2, we fixed the molar concentration of tertiary amines at 6.75 mM instead of weight concentration of polymers. The corresponding weight concentrations of PEO_{114}-*b*-$PDPA_5$, PEO_{114}-*b*-$PDPA_{10}$, PEO_{114}-*b*-$PDPA_{20}$ and PEO_{114}-*b*-$PDPA_{60}$ and PEO_{114}-*b*-$PDPA_{100}$ were 7.6, 4.8, 3.2, 2.0 and 1.8 mg/ml, respectively.

Fluorescence characterization

The fluorescence emission spectra were obtained on a Hitachi fluorometer (F-7500 model, Japan). For each copolymer, the sample was initially prepared in Milli-Q water at the concentration of 2 mg/mL. Then the stock solution was diluted in 0.2 M sodium phosphate buffers (containing 0.15 M sodium chloride) at different pH values. The TMR conjugated nanoprobes were excited at 545 nm, and the emission spectra were collected from 560 to 750 nm. The emission and excitation slits were both 5 nm. The emission intensity at 580 nm was used to quantify the ultra-pH response for different nanoprobe concentrations as shown in Figure 7d.

Measurement of critical micelle concentration (CMC)

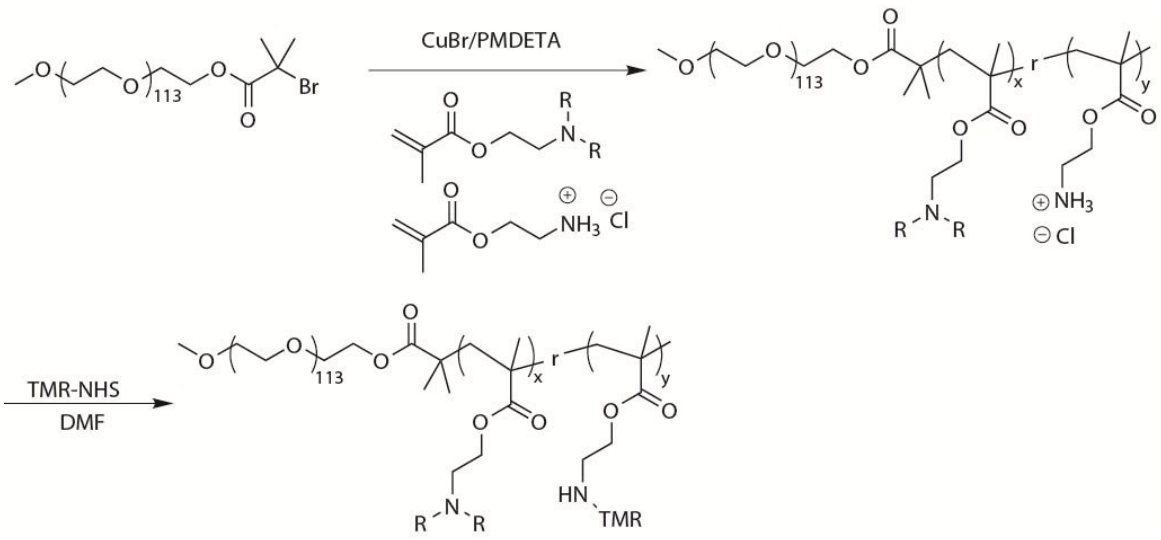
CMC of PEO-b-nPDPA block copolyemrs was measured in 0.1 M phosphate buffer saline (PBS, pH 7.4). First, a copolymer stock solution (5 mg/mL) was diluted to different concentrations in the same buffer. In each solution, 5μL pyrene in THF solution (2×10^{-4} M) was added to 2 mL polymer solution to produce the final pyrene concentration at 5×10^{-7} M. The fluorescence spectra were recorded on a Hitachi fluoremeter (F-7500 model) with the excitation wavelength of 339 nm and the excitation and emission slits at 10.0 nm and 1.0 nm, respectively. The I_1 and I_3 values were measured as the maximum emission intensity at ca. 372 and 382 nm, respectively. I_1/I_3 ratio was plotted as a function of polymer concentration at different pH values. I_1/I_3 ratio reflects the polarity of the pyrene environment where partition of pyrene in the hydrophobic micelle core leads to decreased I_1/I_3 values.

TEM and DLS characterization

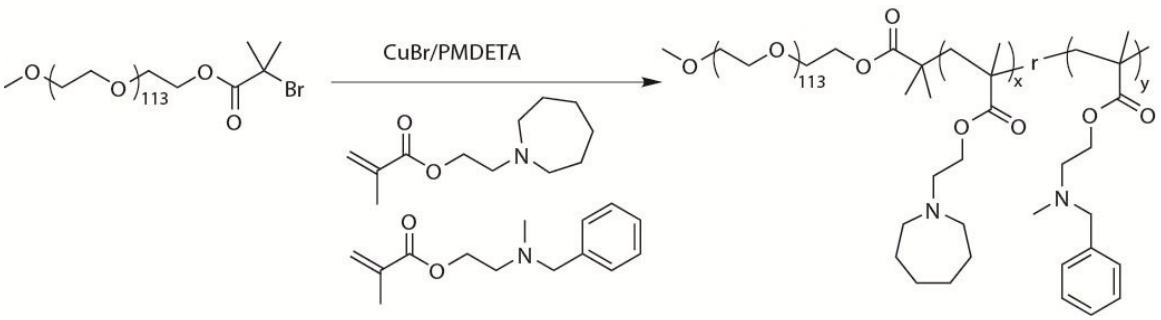
Samples for TEM and DLS analyses were prepared in situ by pH titration. The morphology and size of nanoparticles were characterized by transmission electron microscopy (TEM, FEI Tecnai G2 Spirit Biotwin model). Hydrodynamic diameter (Dh) and scattering count rates were determined by dynamic light scattering (DLS, Malvern Nano-ZS Model, He-Ne Laser, λ=633 nm).

References

1. Bronstein, L.M.*, et al.* Metalated diblock and triblock poly (ethylene oxide)-block-poly (4-vinylpyridine) copolymers: Understanding of micelle and bulk structure. *The Journal of Physical Chemistry B* **109**, 18786-18798 (2005).
2. Zhou, K.*, et al.* Tunable, Ultrasensitive pH‐Responsive Nanoparticles Targeting Specific Endocytic Organelles in Living Cells. *Angewandte Chemie International Edition* **50**, 6109-6114 (2011).
3. Nasongkla, N.*, et al.* Multifunctional polymeric micelles as cancer-targeted, MRI-ultrasensitive drug delivery systems. *Nano letters* **6**, 2427-2430 (2006).



Scheme S1. Syntheses of TMR-conjugated PEO-*b*-PR block copolymers.



Scheme S2. Syntheses of PEO-*b*-P(C7A-r-MBA) block copolymers.

Table S1. Characterization of PEO-*b*-PR block copolymers with linear or branched alkyl substituents on the tertiary amines.

Copolymer	$M_{w,GPC}$ ($\times 10^{-4}$ D)[a]	$M_{n,GPC}$ ($\times 10^{-4}$ D)[a]	PDI[a]	Repeating units	$M_{n,1H\ NMR}$ (x 10^{-4} D)[b]
PEO-*b*-iPDPA	2.32	1.93	1.14	85	2.31
PEO-*b*-nPDPA	2.48	2.05	1.21	80	2.20
PEO-*b*-PDBA	2.79	2.35	1.19	73	2.26
PEO-*b*-PD5A	3.12	2.61	1.20	83	2.73

[a] Number-average (M_n), weight-average molecular weight (M_w) and polydispersity index (PDI) (PDI=M_w/M_n) were determined by GPC using THF as the eluent. [b] Determined by ^{1}H NMR.

Table S2. Characterization of PEO-*b*-nPDPA block copolymers with different hydrophobic chain length.

Copolymer	$M_{w,GPC}$ ($\times 10^{-4}$ D)[a]	$M_{n,GPC}$ ($\times 10^{-4}$ D)[a]	PDI[a]	Repeating units	$M_{n,1H\ NMR}$ (x 10^{-4} D)[b]
PEO-*b*-nPDPA$_5$	0.87	0.66	1.32	6	0.63
PEO-*b*-nPDPA$_{10}$	1.07	0.83	1.29	10	0.72
PEO-*b*-nPDPA$_{20}$	1.32	1.09	1.22	22	0.98
PEO-*b*-nPDBA$_{60}$	2.29	2.01	1.14	64	1.86
PEO-*b*-nPDPA$_{100}$	3.33	2.71	1.23	98	2.59

[a] Number-average (M_n), weight-average molecular weight (M_w) and polydispersity index (PDI) (PDI=M_w/M_n) were determined by GPC using THF as the eluent. [b] Determined by ^{1}H NMR.

Table S3. Characterization of PEO-*b*-PR block copolymers with cyclic alkyl substituents on the tertiary amines.

Copolymer	$M_{w,GPC}$ ($\times 10^{-4}$ D)[a]	$M_{n,GPC}$ ($\times 10^{-4}$ D)[a]	PDI[a]	Repeating units	$M_{n,^1H\ NMR}$ (x 10^{-4} D)[b]
PEO-*b*-PC6A	3.30	2.68	1.23	95	2.37
PEO-*b*-PC6S1A	3.52	2.71	1.30	82	2.23
PEO-*b*-PC6S2A	3.71	2.63	1.41	78	2.26
PEO-*b*-PC7A	3.44	2.69	1.28	86	2.31
PEO-*b*-PMBA	3.78	2.78	1.36	73	2.20

[a] Number-average (M_n), weight-average molecular weight (M_w) and polydispersity index (PDI) (PDI=M_w/M_n) were determined by GPC using THF as the eluent. [b] Determined by ^{1}H NMR.

Table S4. Characterization of PEO-*b*-P(C7A$_x$-r-MBA$_y$) block copolymers with different MBA molar ratio.

Designed MBA molar ratio	x + y	y	y/(x+y)
10%	75	7	9.3%
20%	69	13	19%
30%	63	20	32%
40%	66	25	38%

Determined by ^{1}H-NMR.

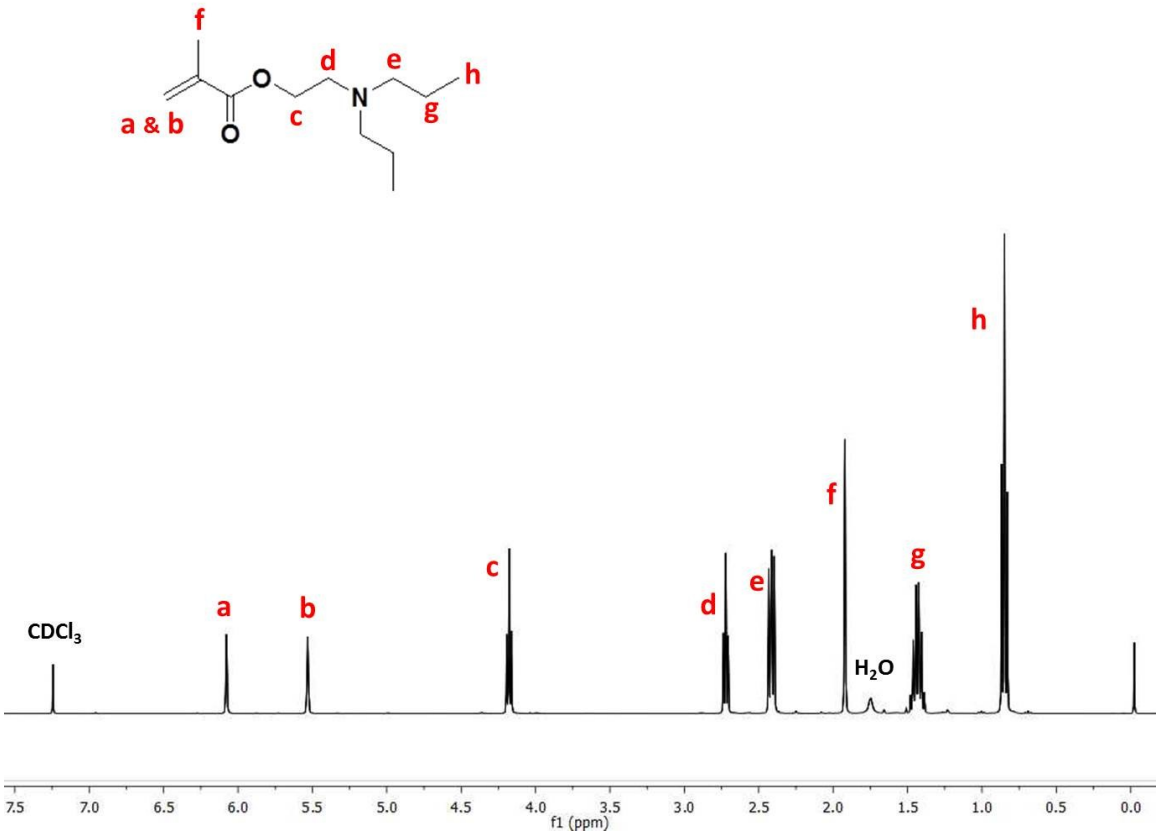


Figure S1. ^{1}H NMR spectra of a representative monomer DPA-MA in CD_3Cl.

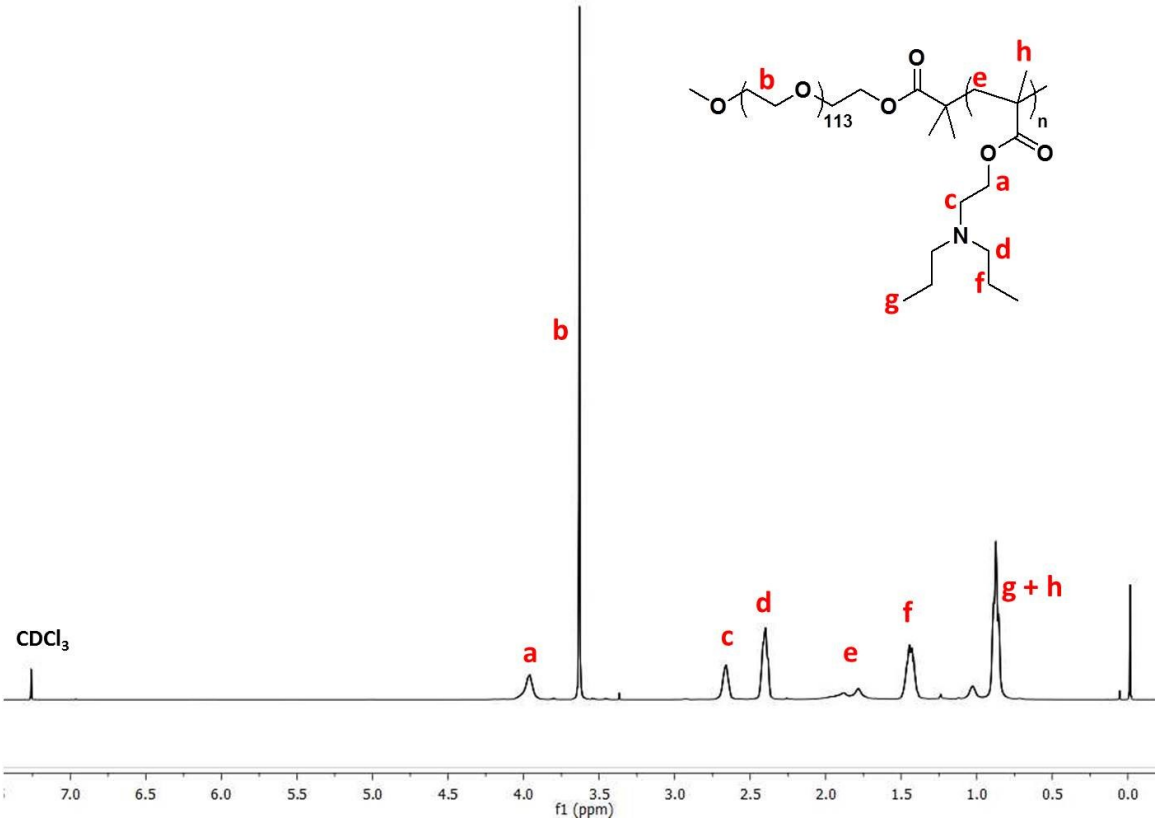


Figure S2. ^{1}H NMR spectra of a representative copolymer PEO-*b*-nPDPA in CD_3Cl. $CDCl_3$ is a good solvent for both PEO and nPDPA blocks. Resonance peaks of PEO methylene protons and PDPA protons were observed.

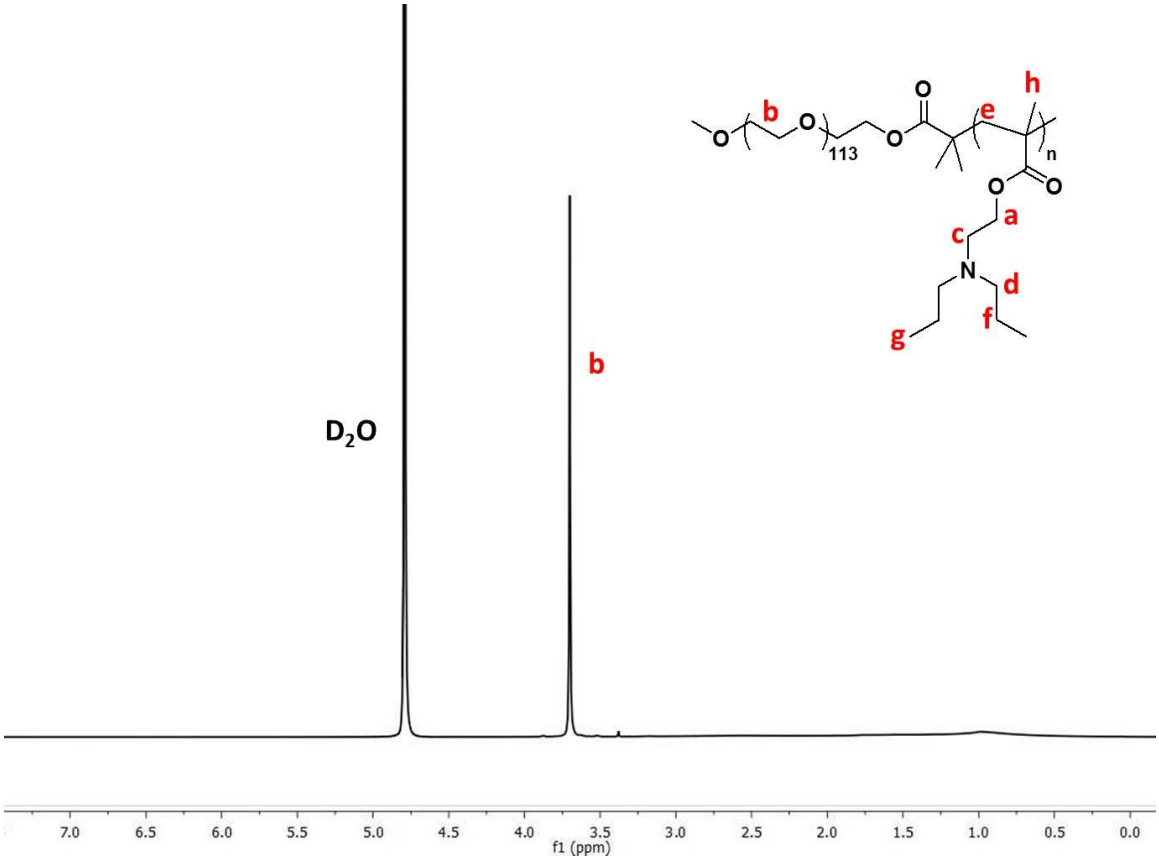


Figure S3. ^{1}H NMR spectra of a representative copolymer PEO-*b*-nPDPA in D_2O. The PEO corona (shells) of micelles were well solvated in D_2O and showed clear ^{1}H NMR signals. In contrast, the resonance peaks of PDPA block were drastically reduced because of their slow mobility in the hydrophobic micellar core.

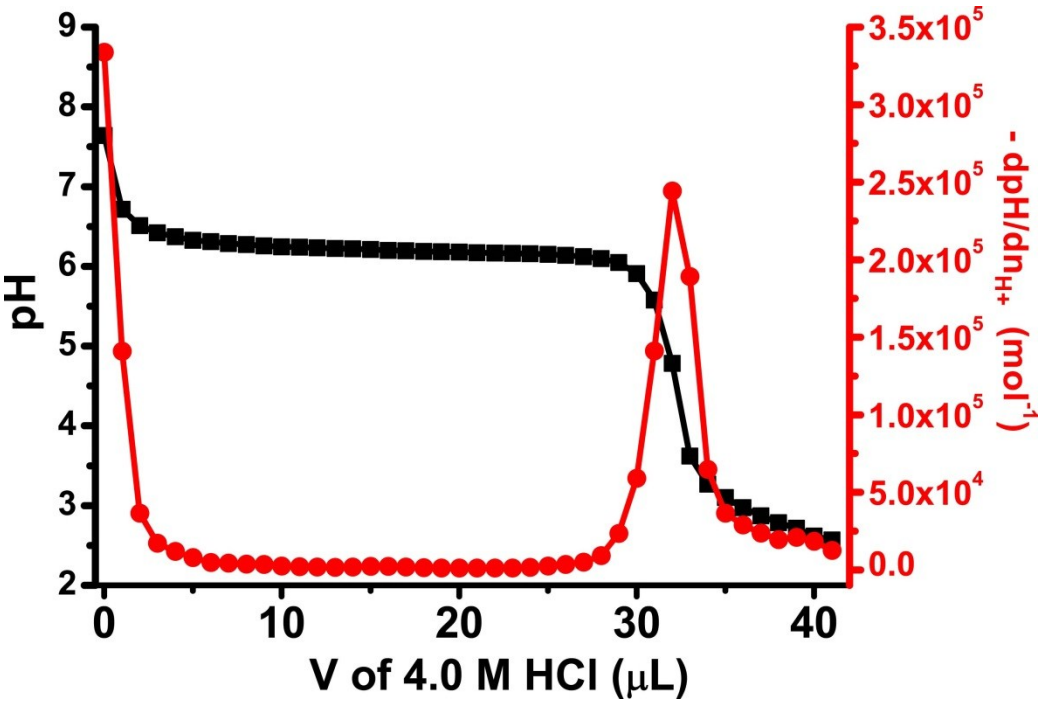


Figure S4. pH titration of PEO-*b*-nPDPA block copolymers. We considered the tertiary amines as 100% protonated when the addition of HCl yielded sharpest change of pH. The calculated $-dpH/dn_{H+}$ was plotted as a function of added HCl. The peak corresponded to the protonation degree at 100% where the amines were 100% converted to ammonium groups.

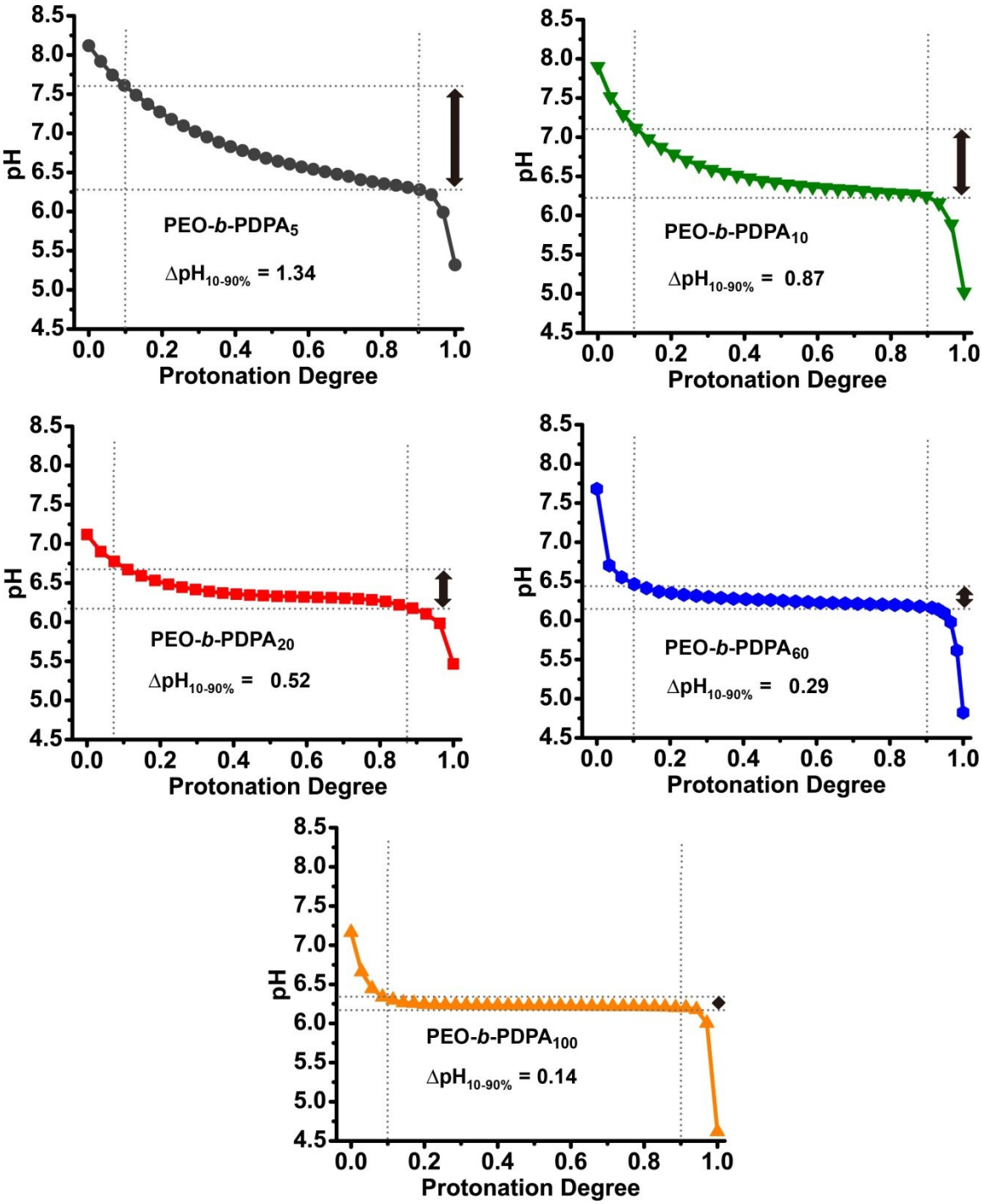


Figure S5. pH transition sharpness ($\Delta pH_{10\text{-}90\%}$) of PEO-*b*-nPDPA block copolymers with different hydrophobic chain lengths.

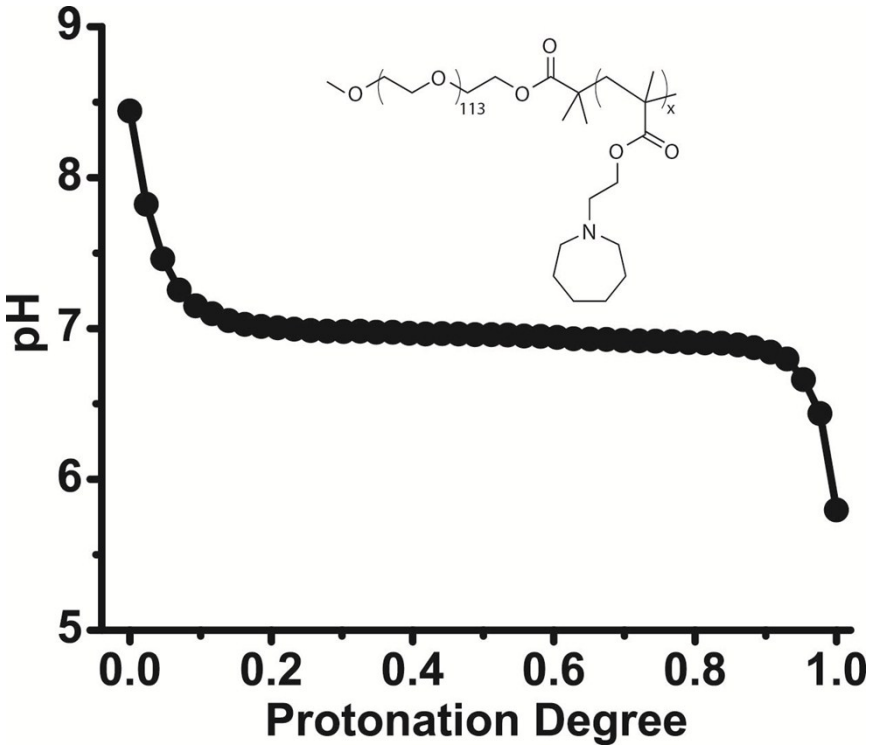


Figure S6. pH titration of PEO-*b*-PC7A block copolymers.

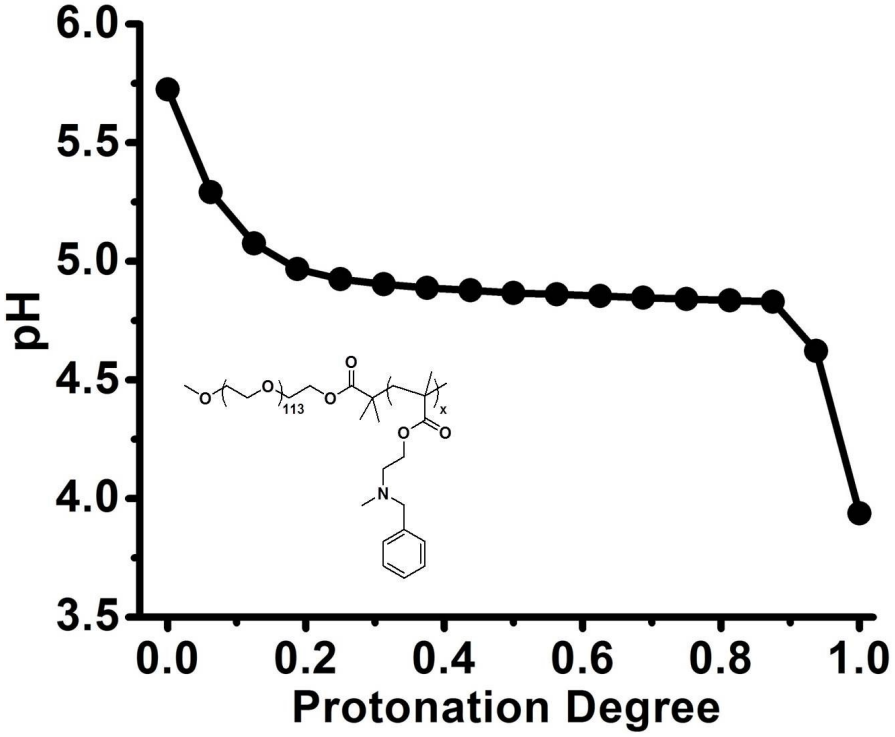


Figure S7. pH titration of PEO-*b*-PMBA (lower panel) block copolymers.

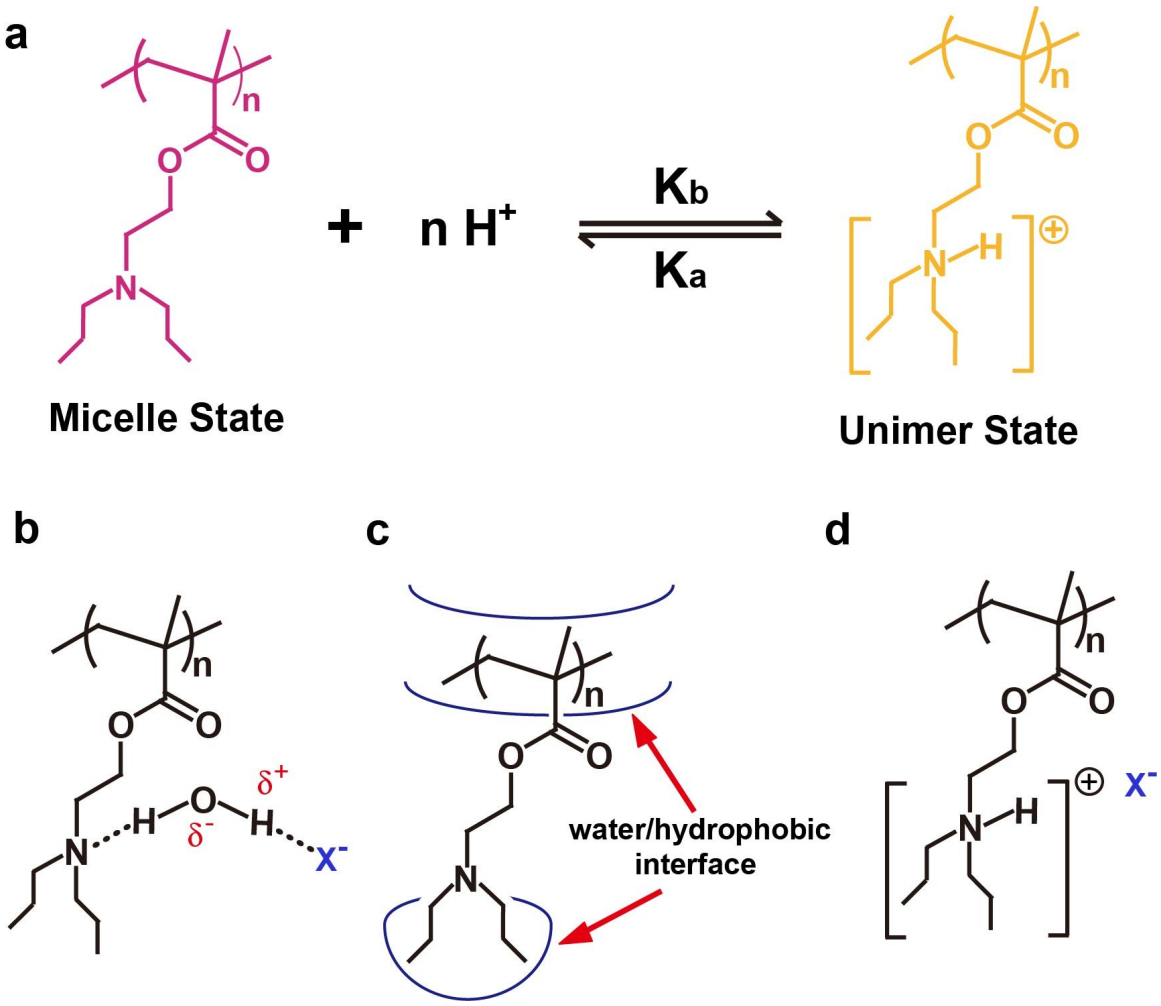


Figure S8. Interactions among anions, PEO-*b*-nPDPA block copolymers and hydration waters. (a) Structural illustration of PEO-*b*-nPDPA block copolymers in unimer or micelle state. For simplification, only hydrophobic PDPA segment was shown. (b) Hydrogen bonding of tertiary amines and hydration water molecules. The hydrogen bonding can be destabilized through polarization by anions, X^-. (c) The hydration of hydrophobic surfaces on PDPA segment is associated with surface tension at the water/ hydrophobic interface. The hydration of hydrophobic surfaces can also be modified by anions, X^-. (d) Direct ion pair interactions between protonated ammonium groups and chaotropic anions.

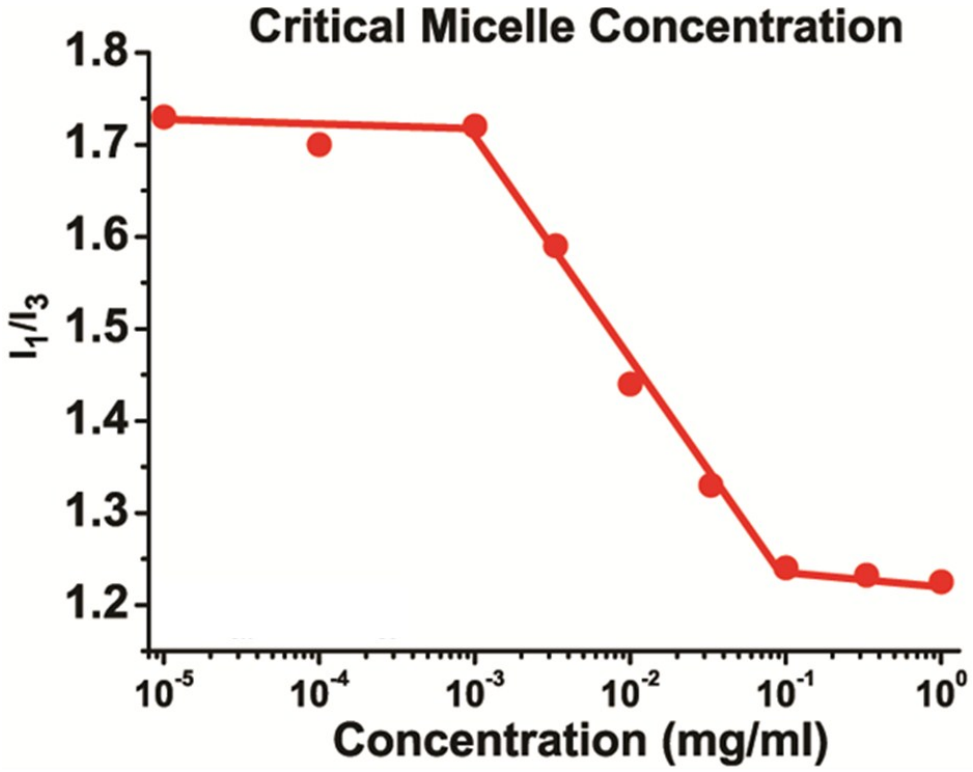


Figure S9. The critical micelle concentration (CMC) of PEO_{114}-*b*-$PDPA_{60}$ block copolymer determined from the measurements of UV absorption of pyrene in polymer solution.

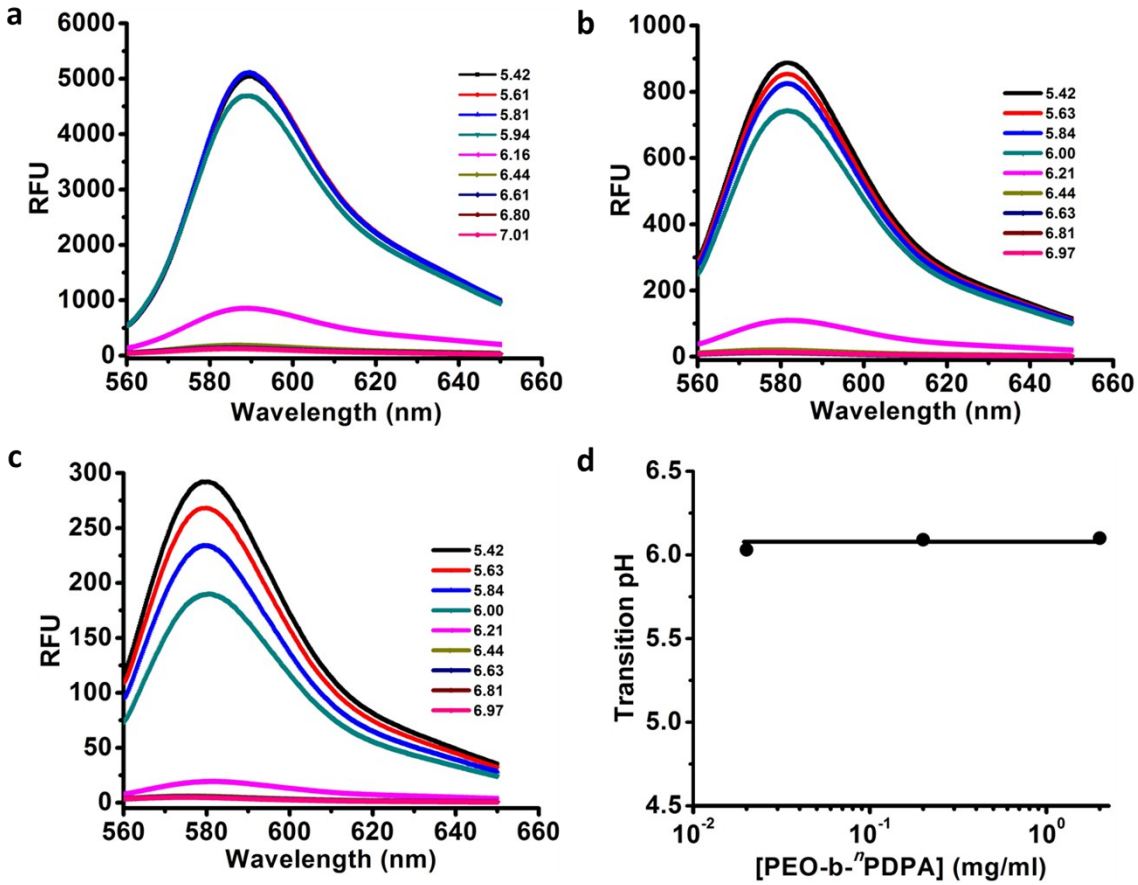


Figure S10. Fluorescence intensity of PEO-*b*-nPDPA-TMR block copolymers at different polymer concentrations in sodium phosphate buffer at pH=7.4: (a) 2.0 mg/ml; (b) 0.2 mg/ml; (c) 0.02 mg/ml. (d) Fluorescence on/off transition pH of TMR conjugated PEO-*b*-nPDPA block copolymers at different polymer concentrations (0.02 to 2.0 mg/ml).