Wide-Range pK_a Tuning of Proton Imprinted Nanoparticles for Reversible Protonation of Target Molecules via Thermal Stimuli

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Supporting Information

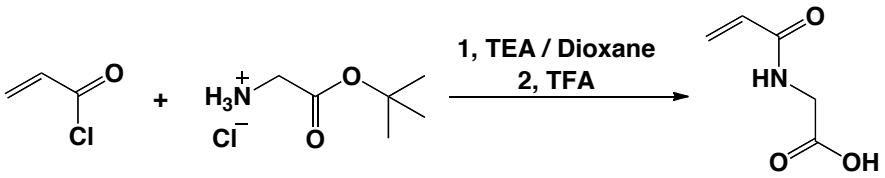
I. Materials

The following chemicals and materials were obtained from commercial sources and used as received. *N*,*N*′-methylenebisacrylamide (BIS), methacrylic acid (MAc), triethyl amine (TEA), and acryloyl chloride were obtained from Tokyo Chemical Industry Co., Ltd. HCl, hexane, sodium chloride, acrylic acid (AAc), sodium dodecyl sulfate (SDS), trifluoromethyl acrylic acid (TfMAAc), 1,4-dioxane, trifluoroacetic acid (TFA), 4,4'-azobis(4-cyanovaleric acid) (V-501), glycine *t*-butyl ester hydrochloride, and L-valine *t*-butyl ester hydrochloride were obtained from Watanabe Pure Chemical Industry Co., Ltd. NaOH was obtained from Kanto Chemical Co., Inc.; α-chloroacrylic acid (CAc) was obtained from Gute Chemie Co., Ltd. *N*-isopropylacrylamide (NIPAm) was obtained from Wako Pure Chemical Industries, Co., Ltd. and recrystallized from hexanes. The dialysis tube (MWCO 12,000–14,000 Da, Spectrum Laboratories, Inc.) was washed with water prior to use. Cation exchange beads (Muromac C1002-H, Muromachi Chemicals, Inc.) were pretreated with aqueous HCl (1 M) and then washed with excess water. The water used in this study was purified using a Direct-Q Ultrapure Water System (Merck, Ltd.).

II. Synthesis of *N*-acryloyl glycine

To synthesize *N*-acryloyl glycine, *N*-acryloylglycine *t*-butyl ester was synthesized first through the condensation reaction of acryloyl chloride and glycine *t*-butyl ester hydrochloride, followed by deprotection of the *t*-butyl group.

Scheme S1.



Synthesis of N-acryloylglycine t-butyl ester

t-Butyl glycine chloride (3.4 g, 20 mmol) was suspended in 80 mL of 1,4-dioxane and 9.2 mL (66 mmol) of triethylamine (TEA) in a 200 mL round-bottom flask containing a magnetic stir bar and cooled on ice. Acryloyl chloride (4.8 mL, 60 mmol) was added dropwise to the suspension on ice over 5 min. After warming to room temperature, the mixture was stirred for 1 h. *N*-Acryloylglycine *t*-butyl ester was extracted by ethyl acetate once and washed with water twice. Water in the organic phase was removed with $MgSO_4$, and the solution was dried in vacuo. *N*-Acryloylglycine *t*-butyl ester was purified by silica-gel chromatography (ethyl acetate:hexane = 1:1). The solvent was evaporated and the product was dried in vacuo overnight (Yield: 39%, 1.4 g). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.43 (d, 1H), 6.27 (dd, 1H), 6.10 (dd, 1H), 5.62 (dd, 1H), 3.81 (d, 2H), 1.41 (m, 9H).

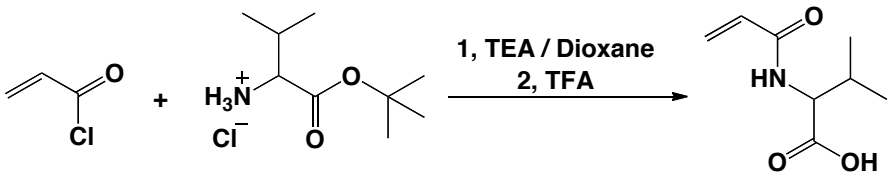
Deprotection of N-acryloylglycine t-butyl ester

To 460 mg of *N*-acryloylglycine *t*-butyl ester (2.5 mmol) was added 5.0 mL of TFA. The mixture was stirred for 15 min at room temperature. The TFA was evaporated under reduced pressure to give an oil that was then mixed with toluene and evaporated twice to remove any residual TFA. The product was dried in vacuo overnight. (Yield: ~100%). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.43 (d, 1H), 6.29 (dd, 1H), 6.10 (dd, 1H), 5.64 (dd, 1H), 3.83 (d, 2H).

III. Synthesis of *N*-acryloyl L-valine

To synthesize *N*-acryloyl L-valine, *N*-acryloyl L-valine *t*-butyl ester was synthesized first through the condensation reaction of acryloyl chloride and L-valine *t*-butyl ester hydrochloride, followed by deprotection of the *t*-butyl group.

Scheme S2.



Synthesis of N-acryloyl L-valine t-butyl ester

t-Butyl L-valine chloride (4.2 g, 20 mmol) was suspended in 80 mL of 1,4-dioxane and 9.2 mL (66 mmol) of triethylamine (TEA) in a 200 mL round-bottom flask containing a magnetic stir bar and cooled on ice. Acryloyl chloride (3.2 mL, 40 mmol) was added dropwise to the suspension on ice over 5 min. After warming to room temperature, the mixture was stirred for 1 h. *N*-acryloyl L-valine *t*-butyl ester was extracted by ethyl acetate once and washed with water twice. Water in the organic phase was removed with $MgSO_4$, and the solution was dried in vacuo. *N*-Acryloyl L-valine *t*-butyl ester was purified by silica-gel chromatography (ethyl acetate:hexane = 1:5 to 1:2). The solvent was evaporated and the product was dried in vacuo overnight (Yield: 33%, 1.5 g). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.24 (d, 1H), 6.43 (dd, 1H), 6.10 (dd, 1H), 5.62 (dd, 1H), 4.18 (dd, 1H), 2.05 (ddd, 1H), 1.41 (s, 9H), 0.88 (m, 6H).

Deprotection of N-acryloyl L-valine t-butyl ester

To 150 mg of *N*-acryloyl L-valine *t*-butyl ester (0.63 mmol) was added 5.0 mL of TFA. The mixture was stirred for 15 min at room temperature. The TFA was evaporated under reduced pressure to give an oil that was then mixed with toluene and evaporated twice to remove any residual TFA. The product was dried in vacuo overnight (Yield: ~100%). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.22 (d, 1H), 6.43 (dd, 1H), 6.10 (dd, 1H), 5.62 (dd, 1H), 4.25 (dd, 1H), 2.08 (ddd, 1H), 0.88 (m, 6H).

IV. Preparation of nanoparticles (NPs)

NPs were prepared as previously reported.[1] Briefly, NIPAm, BIS, and a variety of acrylic monomers with carboxylic acids were dissolved in 25 mL of water, resulting in a total monomer concentration of 312 mM. Monomers containing carboxylic acids, i.e., AAc, MAc, TfMAAc, CAc, *N*-acryloylglycine, or *N*-acryloyl L-valine, were used in this study. *N*-Acryloylglycine and *N*-acryloyl L-valine were dissolved in a small amount (1 mL) of DMSO (because the solubility of the monomers in water was not very high) then injected into the polymerization solution. The amounts of each of the monomer feeds in the polymerization solutions are shown in Tables S1–S7. SDS (6.21 mM, 45 mg) was dissolved in the polymerization solution in order to control the size of the NPs. The pH of the polymerization solution was monitored by a pH meter and adjusted with an HCl or NaOH solution as needed. Nitrogen was bubbled through the reaction mixture for 30 min prior to the start of the polymerization reaction. Following the addition of V-501 (5 mg/0.5 mL DMSO), the polymerization was carried out at 70 °C for 3 h under a nitrogen atmosphere. The polymerized solution was purified by dialysis against an excess amount of water (changed at least 3 times a day) for 3 days. Traces of counter-anions were removed by incubating the NP solutions with strong cation exchange beads for 30 min, which were filtered out after the exchange process. The concentration of the NP solution was determined by measuring the weight of the NPs after lyophilization of 1 mL of the ion-exchanged solution. The yields of the NPs are shown in Tables S1–S7.

V. Quantification of hydrodynamic diameter

The hydrodynamic diameters of the NPs were measured with a dynamic light-scattering (DLS) instrument (Zetasizer Nano, Malvern Instruments, Ltd.) using the purified NPs. Solutions were equilibrated at each temperature for 2 min prior to taking the measurements.

VI. pH measurement of NP solutions

The temperature and pH of the solutions were measured every 3 sec by a pH meter (SevenMulti™, Mettler-Toledo) equipped with pH probes (InLab® Routine Pro, Mettler-Toledo) and recorded in situ by a personal computer. The pH probes were calibrated with three standard buffers (pH 7.0, 4.0, and 9.2) prior to use. The temperature of the solution was controlled by a magnetic stirrer equipped with a thermostat (PPW-2000, Tokyo Rikakikai Co., Ltd.) that was connected with two cryogenic cooling units (CCA-1110 and CCA-1111, Tokyo Rikakikai Co., Ltd.).

VII. Acid-base titration

The NP solution (4 mg/mL, ~2 mM) was titrated with 0.1 M NaOH at 30 °C and 75 °C with stirring at 500 rpm. The temperature of the NP solution was controlled using a water bath. The pK_a values of the carboxylic acids in each NPs were determined by the Henderson–Hasselbalch equation. We first triplicated the titration experiment and confirmed that experimental error of pK_a value acquired from the titration was very small (<0.03) (Fig. S12).

VIII. Reversible protonation of target molecules

NPs were dissolved in pure water. The pH of the NP solution was adjusted to the pK_a of each NP by addition of an aqueous solution of NaOH. The concentration of each NP solution was adjusted to 4 mg/mL. MR or BTB (0.5 mM) was dissolved in the solution. The color of the solution at 30 °C and 75 °C was recorded on an iPhone.

Table S1. Polymerization conditions and yield of AAc-NPs polymerized at different pH values.

Polymerization pH	Added ions HCl or NaOH/(μmol)	NIPAm feed (mol%)/(mg)	AAc feed (mol%)/(μL)	BIS feed (mol%)/(mg)	Yield of NPs (%)/(mg)
1.8	HCl/200	93/821	5/27	2/24	74/629
2.3	HCl/50	93/821	5/27	2/24	95/802
2.6	Not added	93/821	5/27	2/24	99/833
3.5	NaOH/100	93/821	5/27	2/24	100/848
4.3	NaOH/410	93/821	8/43	2/24	102/866
5.3	NaOH/690	93/821	10/53	2/24	87/737
9.1	NaOH/760	93/821	10/53	2/24	75/631

Table S2. Polymerization conditions and yield of MAc-NPs polymerized at different pH values.

Polymerization pH	Added ions HCl or NaOH/(μmol)	NIPAm feed (mol%)/(mg)	MAc feed (mol%)/(μL)	BIS feed (mol%)/(mg)	Yield of NPs (%)/(mg)
2.2	HCl/200	93/821	5/33	2/24	73/641
3.1	Not added	93/821	5/33	2/24	78/684
4.0	NaOH/120	93/821	5/33	2/24	74/650
4.4	NaOH/220	93/821	5/33	2/24	77/676
5.4	NaOH/390	93/821	5/33	2/24	71/623
6.7	NaOH/420	93/821	5/33	2/24	67/588

Table S3. Polymerization conditions and yield of TfMAAc-NPs polymerized at different pH values.

Polymerization pH	Added ions HCl or NaOH/(μmol)	NIPAm feed (mol%)/(mg)	TfMAAc feed (mol%)/(mg)	BIS feed (mol%)/(mg)	Yield of NPs (%)/(mg)
2.0	HCl/200	93/821	5/55	2/24	82/738
2.3	Not added	93/821	5/55	2/24	82/742
2.8	NaOH 270	93/821	7/81	2/24	77/710
3.4	NaOH 600	93/821	10/105	2/24	78/742
4.2	NaOH 800	93/821	11/120	2/24	76/736
7.0	NaOH 950	93/821	14/156	2/24	66/662

Table S4. Polymerization conditions and yield of CAc-NPs polymerized at different pH values.

Polymerization pH	Added ions HCl or NaOH/(μmol)	NIPAm feed (mol%)/(mg)	CAc feed (mol%)/(mg)	BIS feed (mol%)/(mg)	Yield of NPs (%)/(mg)
1.7	HCl 400 ml	93/821	5/42	2/24	Precipitation
2.1	Not added	93/821	5/42	2/24	82/728
2.6	NaOH 200 ml	93/821	5/42	2/24	83/744
2.9	NaOH 250 ml	93/821	5/42	2/24	77/686
3.3	NaOH 320 ml	93/821	5/42	2/24	80/708
10.1	NaOH 400 ml	93/821	5/42	2/24	72/640

Table S5. Polymerization conditions and yield of glycine-NPs polymerized at different pH values.

Polymerization pH	Added ions HCl or NaOH/(μmol)	NIPAm feed (mol%)/(mg)	Glycine feed (mol%)/(mg)	BIS feed (mol%)/(mg)	Yield of NPs (%)/(mg)
2.0	HCl/200	93/821	6.3/63	2/24	70/635
2.6	Not added	93/821	7.1/71	2/24	67/612
4.1	NaOH/450	93/821	9.2/93	2/24	73/684
4.7	NaOH/420	93/821	7.4/74	2/24	76/672
10.8	NaOH/540	93/821	7.8/79	2/24	74/649

Table S6. Polymerization conditions and yield of valine-NPs polymerized at different pH values.

Polymerization pH	Added ions HCl or NaOH/(μmol)	NIPAm feed (mol%)/(mg)	Valine feed (mol%)/(mg)	BIS feed (mol%)/(mg)	Yield of NPs (%)/(mg)
2.0	HCl/150	93/821	7.5/100	2/24	85/800
2.3	Not added	93/821	6.9/93	2/24	83/774
3.6	NaOH/430	93/821	12.0/160	2/24	69/693
4.1	NaOH/450	93/821	9.9/132	2/24	73/716
4.6	NaOH/590	93/821	11.0/147	2/24	62/616
8.3	NaOH/651	93/821	12.9/173	2/24	83/847

Table S7. Incorporation of acid-monomer, VPTT, diameter, and swelling ratio of AAc-NPs polymerized at different pH values.

Polymerization pH	Incorporation of AAc[a] (mol%)	VPTT (℃)	Hydrodynamic diameter (nm)[b]		Swell ratio[b]
			30 ℃	75 ℃	
1.8	5.1	49	318	87	3.7
2.3	4.8	39	214	72	3.0
2.6	4.8	36	202	69	2.9
3.5	4.5	36	190	67	2.8
4.3	4.8	41	257	75	3.4
5.3	4.4	34	206	85	2.4
9.1	4.7	34	230	98	2.4

Table S8. Incorporation of acid-monomer, VPTT, diameter, and swelling ratio of MAc-NPs polymerized at different pH values.

Polymerization pH	Incorporation of MAc[a] (mol%)	VPTT (℃)	Hydrodynamic diameter (nm)[b]		Swell ratio[b]
			30 ℃	75 ℃	
2.2	3.9	36	185	92	2.0
3.1	4.8	36	215	98	2.2
4.0	4.7	34	185	80	2.3
4.4	4.5	34	163	79	2.1
5.4	4.1	34	142	66	2.1
6.7	4.2	34	120	59	2.0

Table S9. Incorporation of acid-monomer, VPTT, diameter, and swelling ratio of TfMAAc -NPs polymerized at different pH values.

Polymerization pH	Incorporation of TfMAAc[a] (mol%)	VPTT (℃)	Hydrodynamic diameter (nm)[b]		Swell ratio[b]
			30 ℃	75 ℃	
2.0	6.2	39	221	86	2.6
2.3	5.9	36	223	86	2.6
2.8	6.8	36	274	85	3.2
3.4	7.1	49	366	108	3.4
4.2	6.0	36	329	99	3.3
7.0	6.5	36	373	119	3.1

Table S10. Incorporation of acid-monomer, VPTT, diameter, and swelling ratio of CAc-NPs polymerized at different pH values.

Polymerization pH	Incorporation of CAc[a] (mol%)	VPTT (°C)	Hydrodynamic diameter (nm)[b]		Swell ratio[b]
			30 °C	75 °C	
1.7	Precipitation	Precipitation	Precipitation	Precipitation	Precipitation
2.1	5.2	-	268	217	1.2
2.6	5.0	44.0	275	101	2.7
2.9	5.1	44.0	242	76	3.2
3.3	5.2	46.0	213	81	2.6
10.1	5.7	-	342	405	0.8

Table S11. Incorporation of acid-monomer, VPTT, diameter, and swelling ratio of glycine-NPs polymerized at different pH values.

Polymerization pH	Incorporation of Glycine[a] (mol%)	VPTT (°C)	Hydrodynamic diameter (nm)[b]		Swell ratio[b]
			30 °C	75 °C	
2.0	6.3	39	446	307	1.4
2.6	6.8	36	298	78	3.8
4.1	7.5	36	317	97	3.3
4.7	6.3	49	226	82	2.7
10.8	6.7	36	437	49	8.9

Table S12. Incorporation of acid-monomer, VPTT, diameter, and swelling ratio of valine-NPs polymerized at different pH values.

Polymerization pH	Incorporation of Valine[a] (mol%)	VPTT (°C)	Hydrodynamic diameter (nm)[b]		Swell ratio[b]
			30 °C	75 °C	
2.0	5.5	41	274	87	3.1
2.3	5.4	46	256	74	3.4
3.6	7.6	34	222	79	2.8
4.1	5.3	34	225	82	2.7
4.6	6.3	34	207	76	2.7
8.3	7.4	34	186	67	2.8

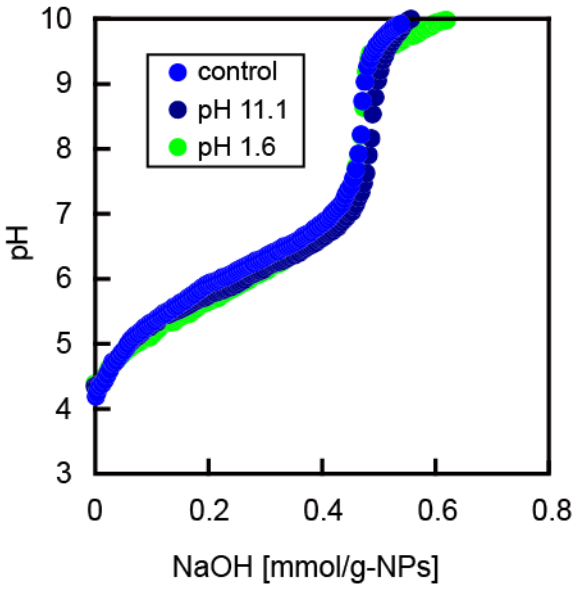


Figure S1. Acid-base titration curves (30 °C) of AAc-NPs before (blue) and after incubated in acidic (green, aqueous HCl solution, pH = 1.8) and basic (dark blue, aqueous NaOH solution, pH = 11.1) condition for 3 hours at 70 °C. The curves show that amount of acids in NPs did not significantly change after incubation, indicating that amide bonds are stable in the acidic and basic polymerization condition.

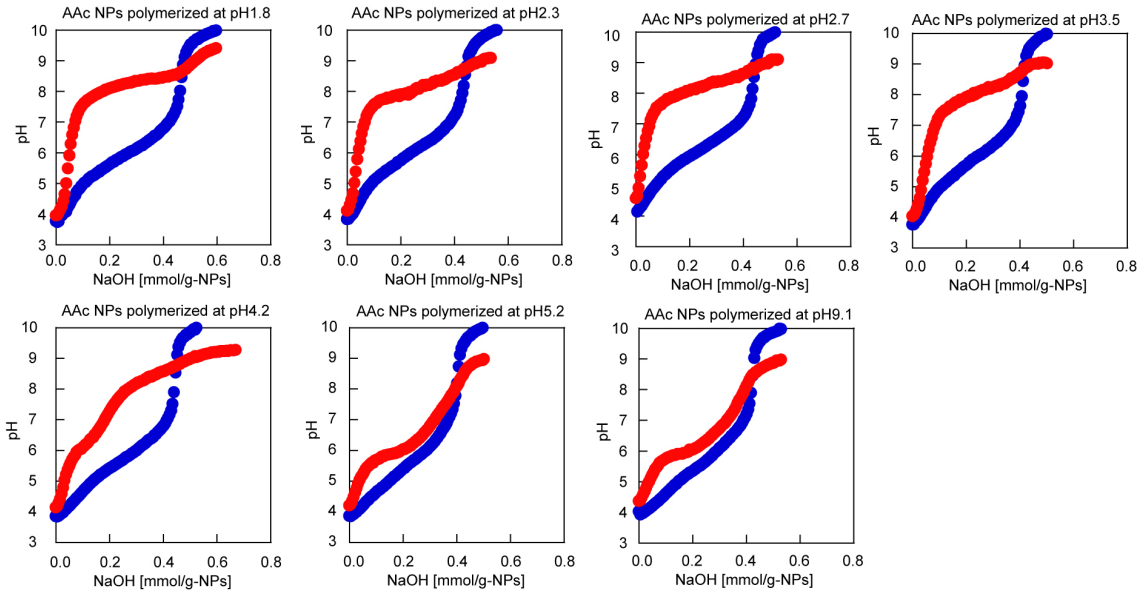


Figure S2. Acid-base titration curves of AAc-NPs polymerized at various pH at 30 °C (blue) and 75 °C (red).

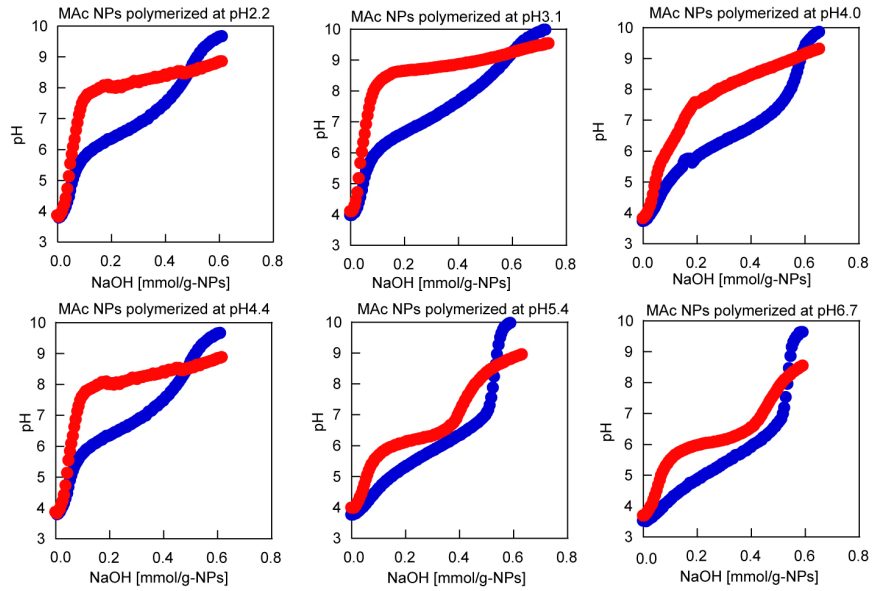


Figure S3. Acid-base titration curves of MAc-NPs polymerized at various pH at 30 °C (blue) and 75 °C (red).

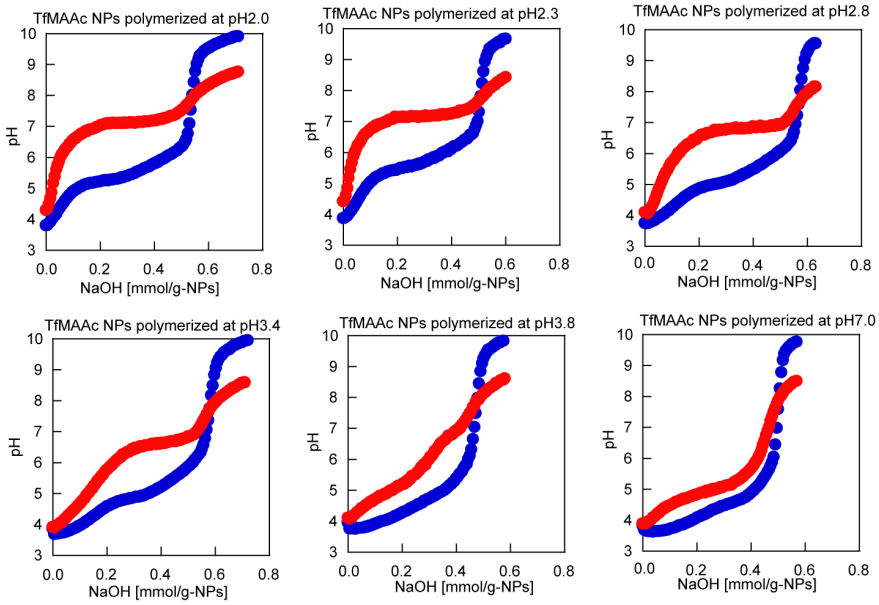


Figure S4. Acid-base titration curves of TfMAAc-NPs polymerized at various pH at 30 °C (blue) and 75 °C (red).

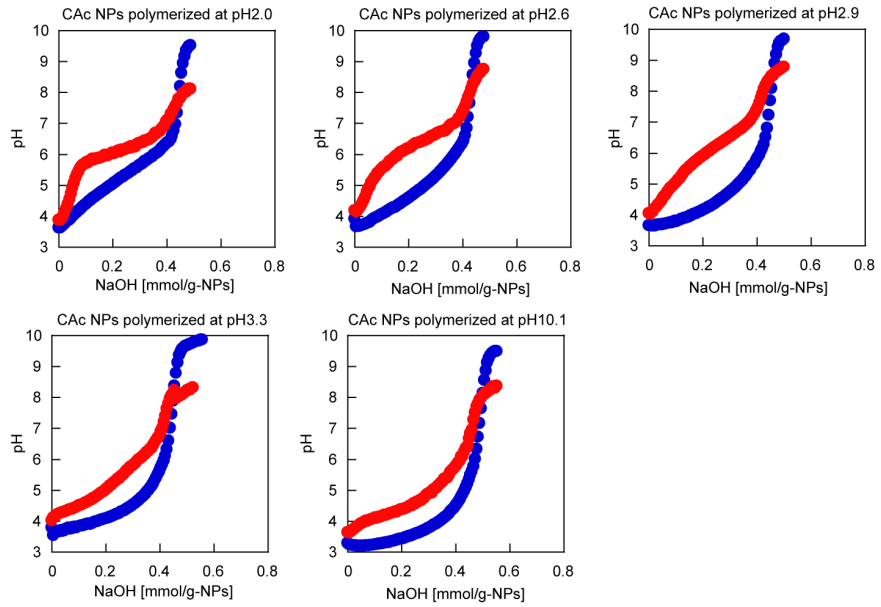


Figure S5. Acid-base titration curves of CAc-NPs polymerized at various pH at 30 °C (blue) and 75 °C (red).

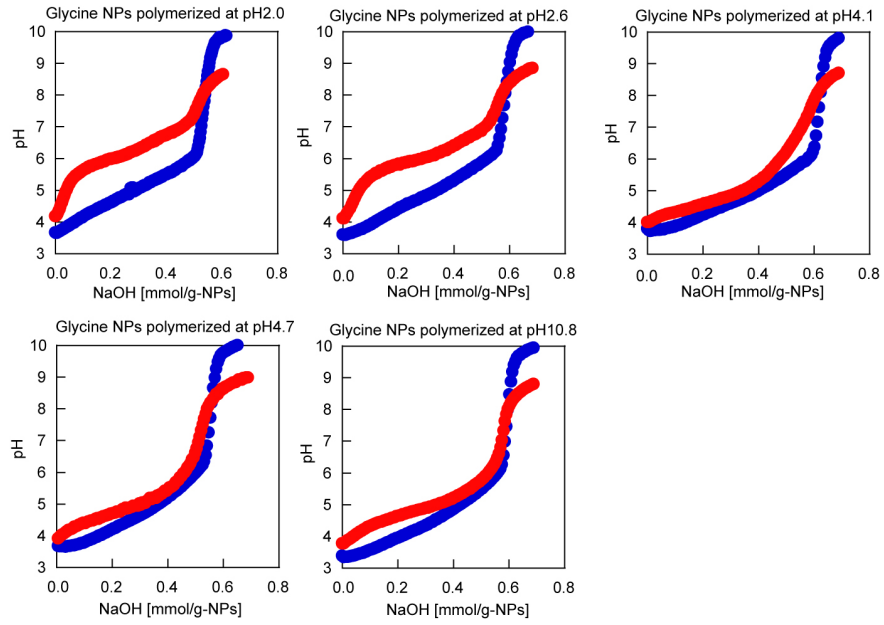


Figure S6. Acid-base titration curves of Glycine-NPs polymerized at various pH at 30 °C (blue) and 75 °C (red).

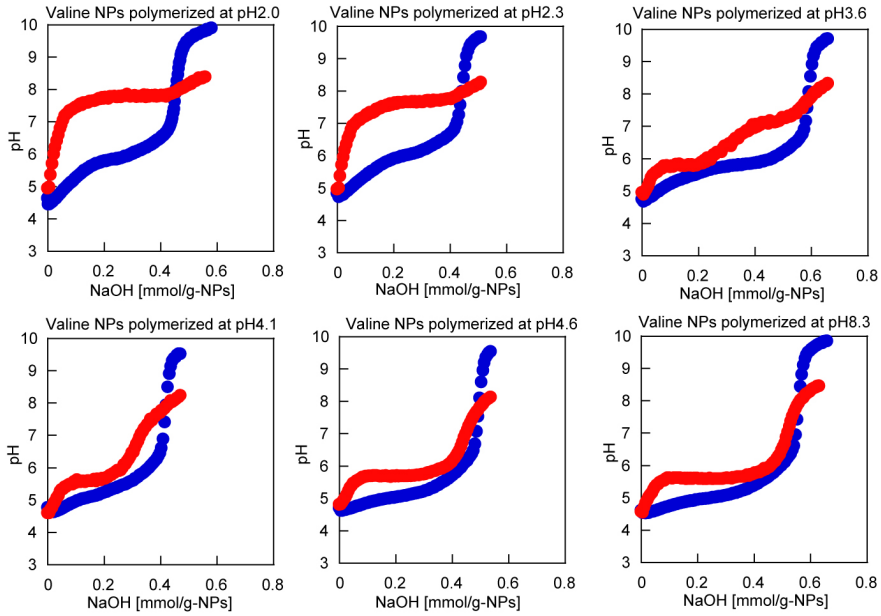


Figure S7. Acid-base titration curves of Glycine-NPs polymerized at various pH at 30 °C (blue) and 75 °C (red).

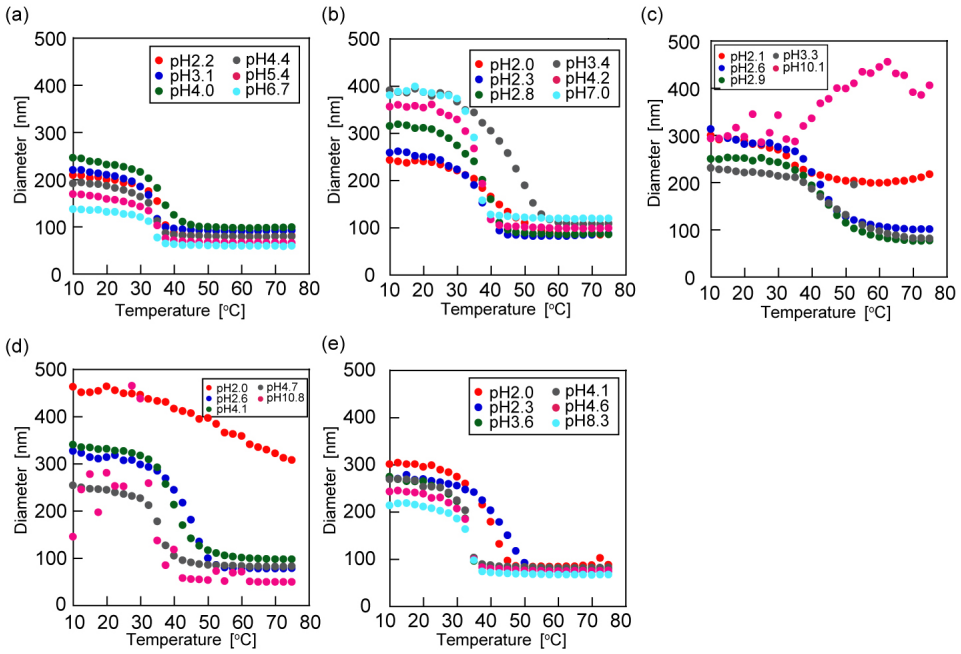


Figure S8. Hydrodynamic diameter of (a) MAc-, (b) TfMAAc-, (c) CAc-, (d) Glycine- and (d)Valine-NPs at 10–80 °C.

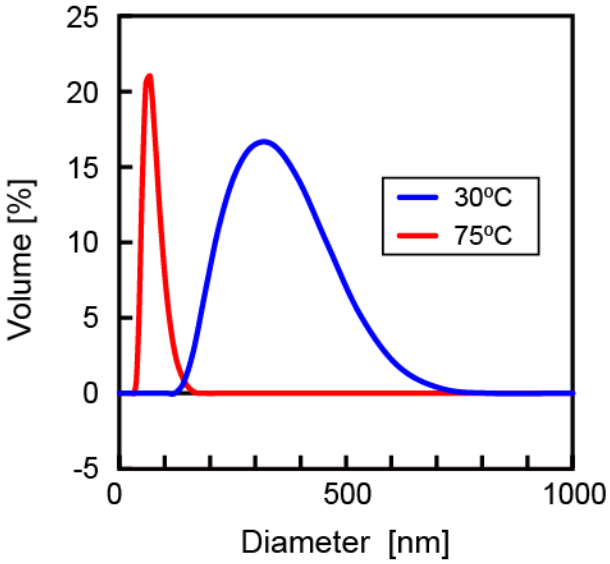


Figure S9. Typical size distribution of AAc-NPs at 30 °C and 75 °C determined by DLS.

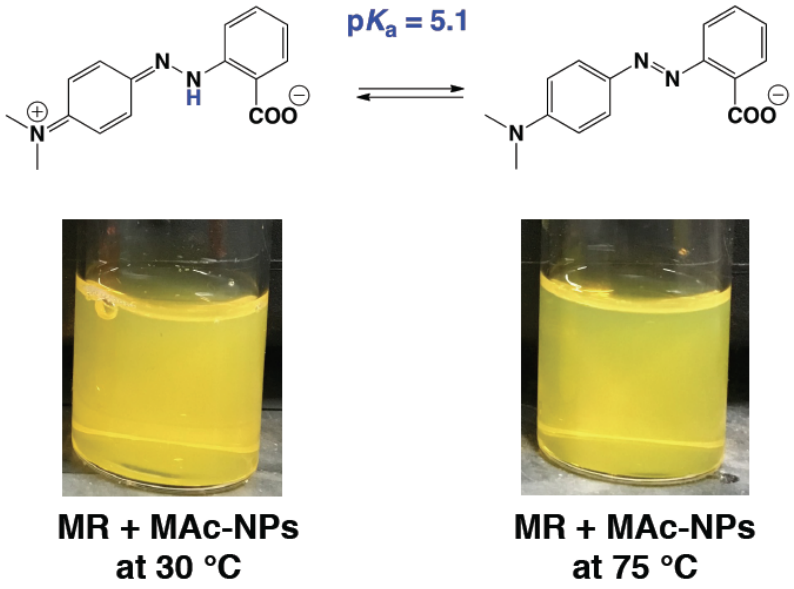


Figure S10. Top: Structure of protonated (left) and deprotonated (right) MR. **Bottom:** the color of MR solutions with MAc-NPs at 30 °C (left) and 75 °C (right). PH of the solution was adjusted to be same as pK_a of MAc NPs at 30 °C (pH = 7.0) prior to the experiment.

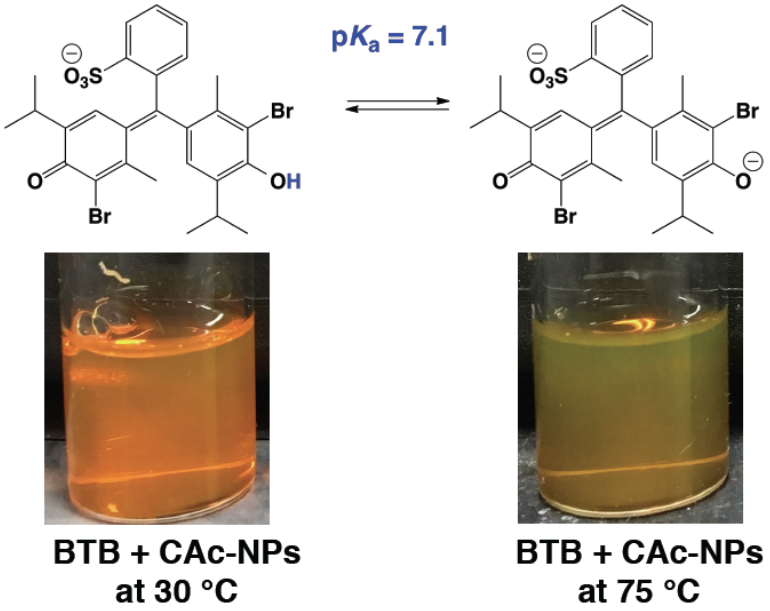


Figure S11. Top: Structure of protonated (left) and deprotonated (right) BTB. **Bottom:** the color of BTB solutions with CAc-NPs at 30 °C (left) and 75 °C (right). PH of the solution was adjusted to be same as pK_a of CAc NPs at 30 °C (pH = 4.3) prior to the experiment.

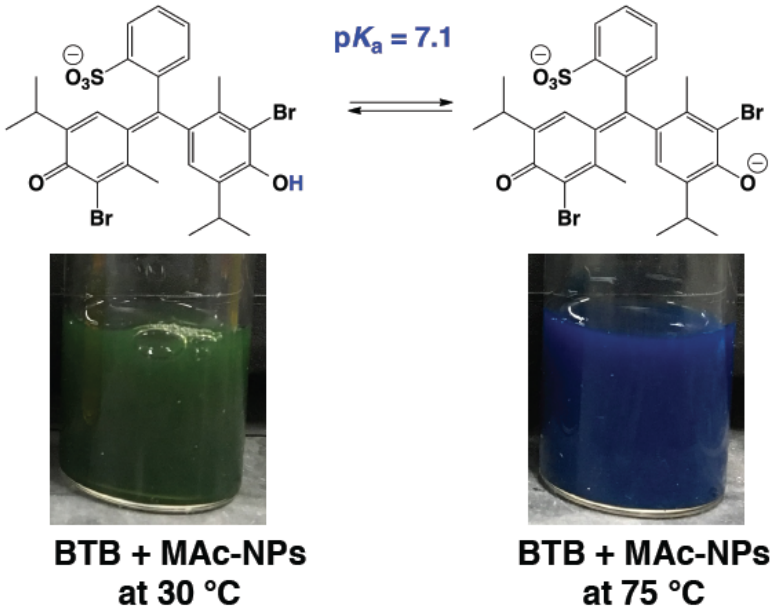


Figure S12. Top: Structure of protonated (left) and deprotonated (right) BTB. **Bottom:** the color of BTB solutions with MAc-NPs at 30 °C (left) and 75 °C (right). PH of the solution was adjusted to be same as pK_a of MAc NPs at 30 °C (pH = 7.0) prior to the experiment.

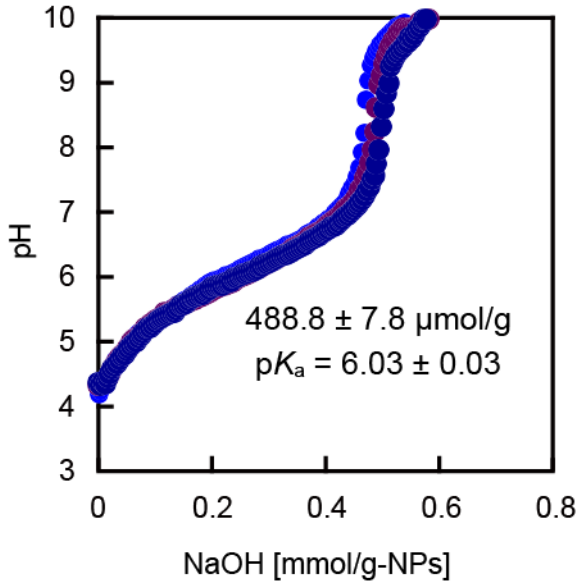


Figure S12. Triprication of Acid-base titration of AAc-NPs at 30 °C.

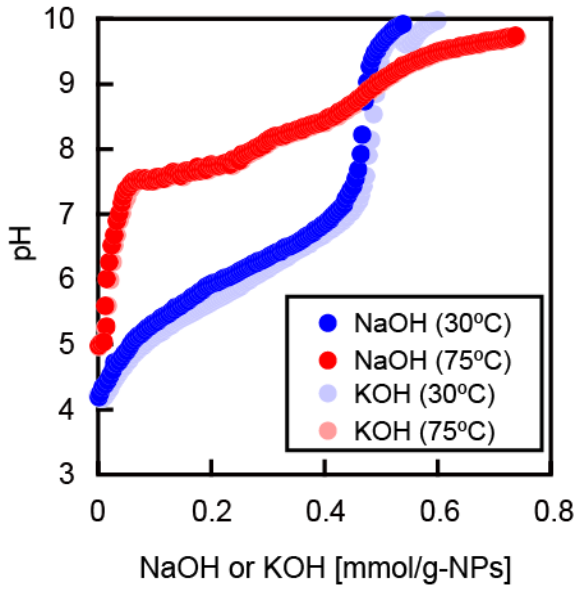


Figure S13. Acid-base titration curves of AAc-NPs polymerized at various pH at 30 °C and 75 °C using NaOH as base (blue and red respectively) and at 30 °C and 75 °C using KOH as base (light blue and right red respectively respectively).