Electronic Supplementary Material (ESI) for RSC Advances.

Supplemental Information

Thermoresponsive Hydrophobic Copolymer Brushes Modified Porous Monolithic Silica for High-Resolution Bioseparation

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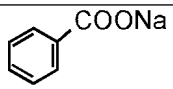
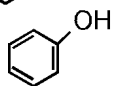
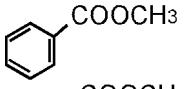
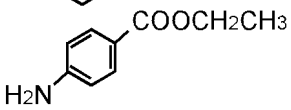
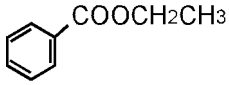
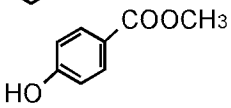
Table S1. Properties of benzoic acids and phenol as used analytes

Compound	Structure	Molecular weight	LogP [a]
Sodium benzoate		144.1	−2.27
Phenol		94.11	1.46
Methyl benzoate		136.15	2.12
Ethyl *p*-aminobenzoate		165.19	1.86
Ethyl benzoate		150.17	2.64
Methyl *p*-hydroxybenzoate		152.15	1.96

a) Partition coefficient in an *n*-octanol/water system.[S1]

Table S2. Amino acid sequences and properties of insulin fragments

Analyte	Amino acid sequence	Molecular weight
Insulin chain A (oxidized)	Gly-Ile-Val-Glu-Gln-Cys(SO_3H)-Cys(SO_3H)-Ala-Ser-Val-Cys(SO_3H)-Ser-Leu-Tyr-Gln-Leu-Glu-Asn-Tyr-Cys(SO_3H)-Asn	2531.64
Insulin chain B (oxidized)	Phe-Val-Asn-Gln-His-Leu-Cys(SO_3H)-Gly-Ser-His-Leu-Val-Glu-Ala-Leu-Tyr-Leu-Val-Cys(SO_3H)-Gly-Glu-Arg-Gly-Phe-Phe-Tyr-Thr-Pro-Lys-Ala	3495.89
Insulin		5807.57

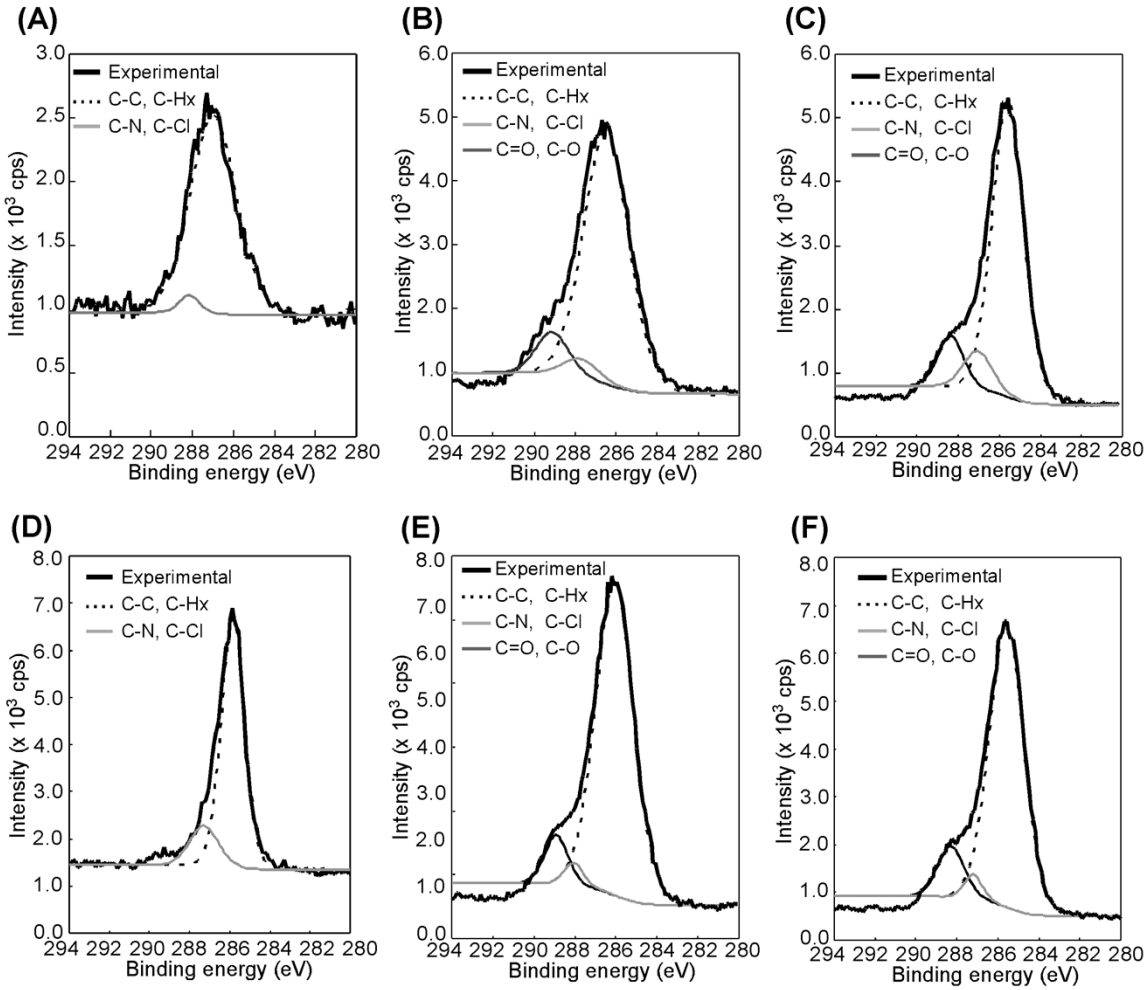


Figure S1. XPS deconvolution of C1s peaks of (A) ATRP-initiator-modified monolithic silica rod (IM in Table 2), (B) short P(IPAAm-*co*-BMA)-brush-grafted monolithic silica rod (IM-4IPB-5), (C) long P(IPAAm-*co*-BMA)-brush-grafted monolithic silica rod (IM-16IPB-5), (D) ATRP-initiator-modified silica beads, (E) short P(IPAAm-*co*-BMA)-brush-grafted silica beads (IB-4IPB-5), and (F) long P(IPAAm-*co*-BMA)-brush-grafted silica beads (IB-16IPB-5). In the spectra of copolymer-grafted monolithic silica and silica bead surfaces, an additional peak was observed at 288 eV, corresponding to the C=O and C–O bonds of the copolymer, whereas there were no such peaks in the spectra of the ATRP-initiator-modified silica surfaces.

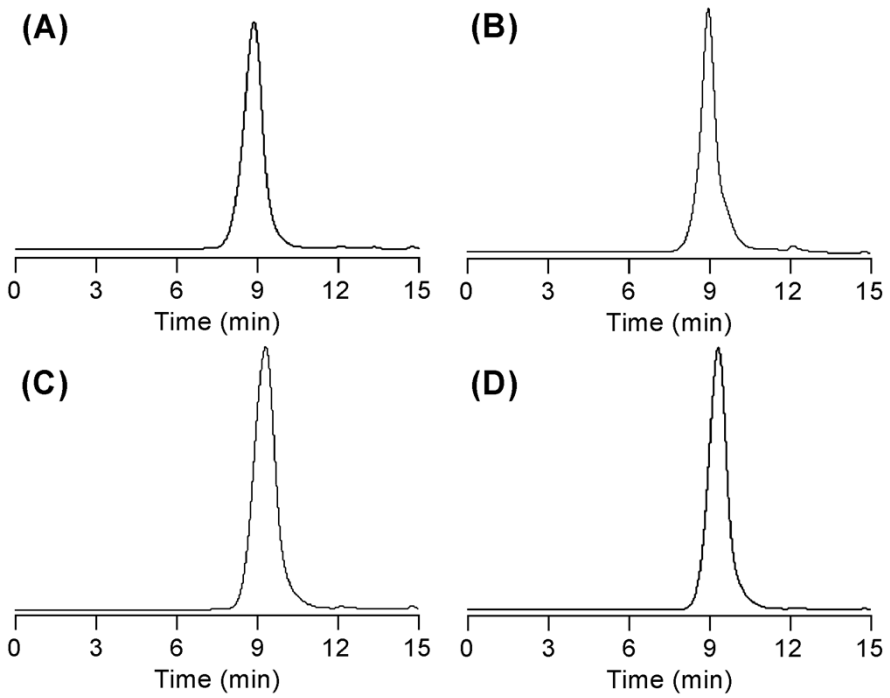


Figure S2. GPC charts of retrieved copolymers used to obtain molecular weights. (A) IM-16IPB-5, (B) IM-4IPB-5, (C) IB-16IPB-5, and (D) IB-4IPB-5. Sample names are explained in Table 2.

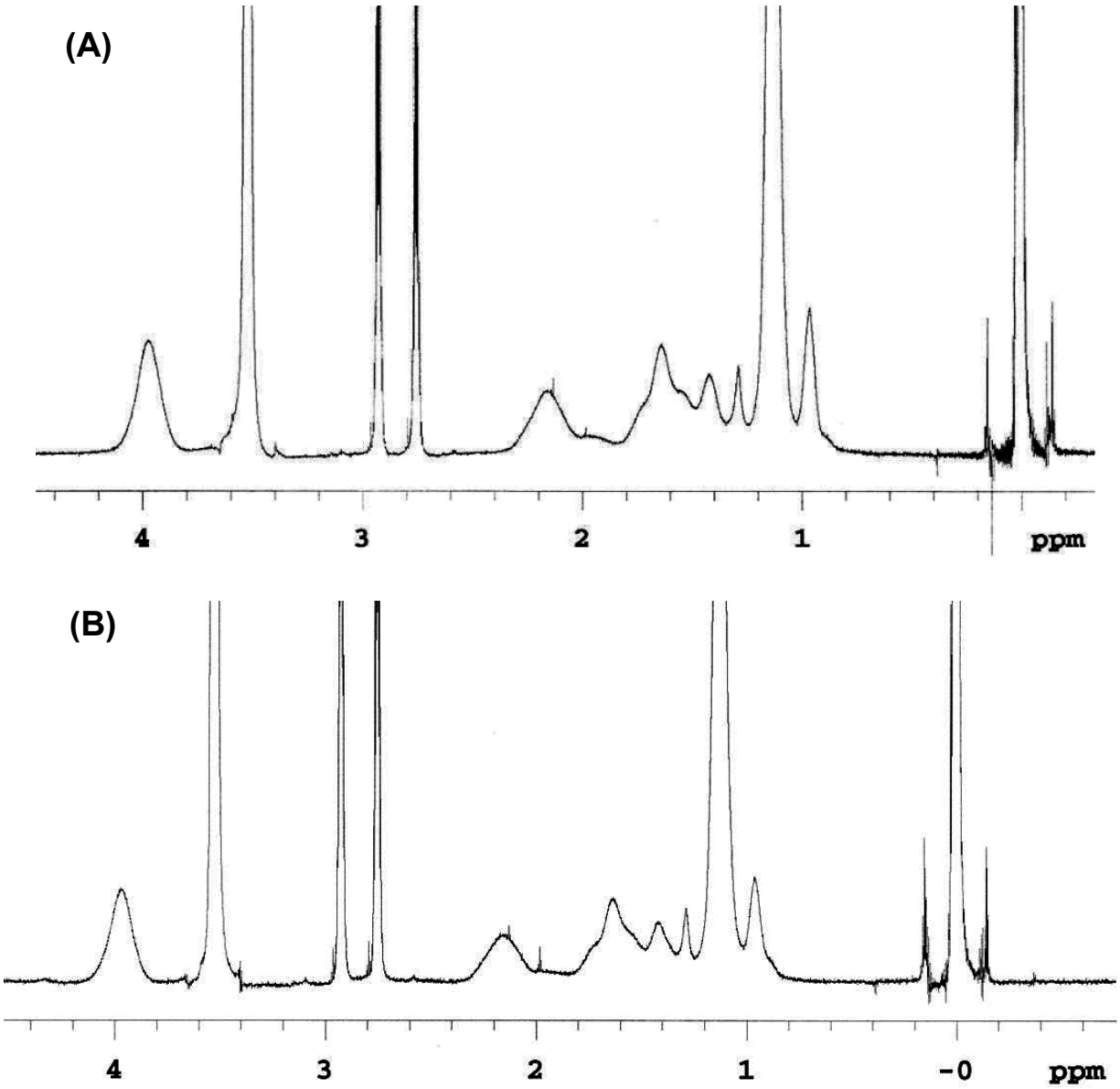


Figure S3. [1]H NMR spectrum of retrieved copolymer from (A) IM-16IPB-5 and (B) IB-16IPB-5. Sample names are explained in Table 2.

Table S3. Characterization of P(IPAAm-*co*-BMA) copolymer retrieved from silica

Code [a)]	IPAAm/BMA (molar ratio)		Peak area at 3.9 ppm	Peak area at 0.9 ppm
	In feed	In copolymer [b)]		
IM-16IPB-5	95.0/5.0	88.15/11.85	3.98	2.53
IB-16IPB-5	95.0/5.0	86.06/13.94	2.52	1.85

a) All samples were named using abbreviated monomer names. "IP" and "B" represent IPAAm and BMA, respectively. b) According to [1]H-NMR results.

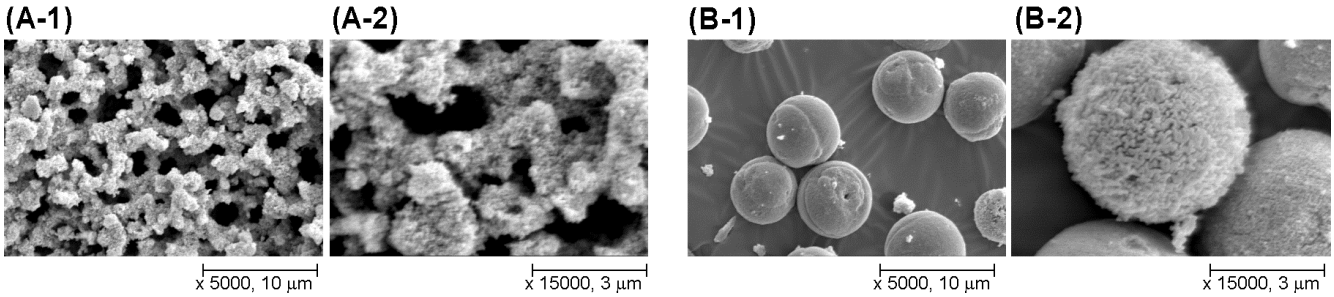


Figure S4. SEM images of monolithic silica rod and silica beads (A-1, 2) ATRP-initiator-modified monolithic silica rod (IM in Table 2), (B-1, 2) ATRP-initiator-modified silica beads (IB in Table 1). A-2 and B-2 (×15 000 magnification) are close-ups of A-1 and B-1 (×5000 magnification), respectively.

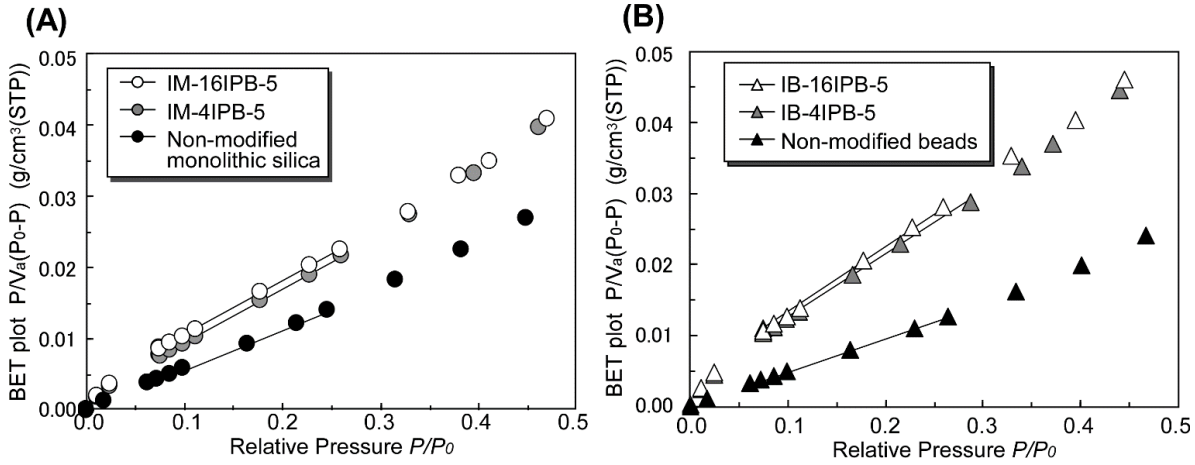


Figure S5. BET plots for the prepared monolithic silica rod (A) and silica beads (B). The silica matrix abbreviations are defined in Table 2, and the obtained surface areas are given in Table 3.

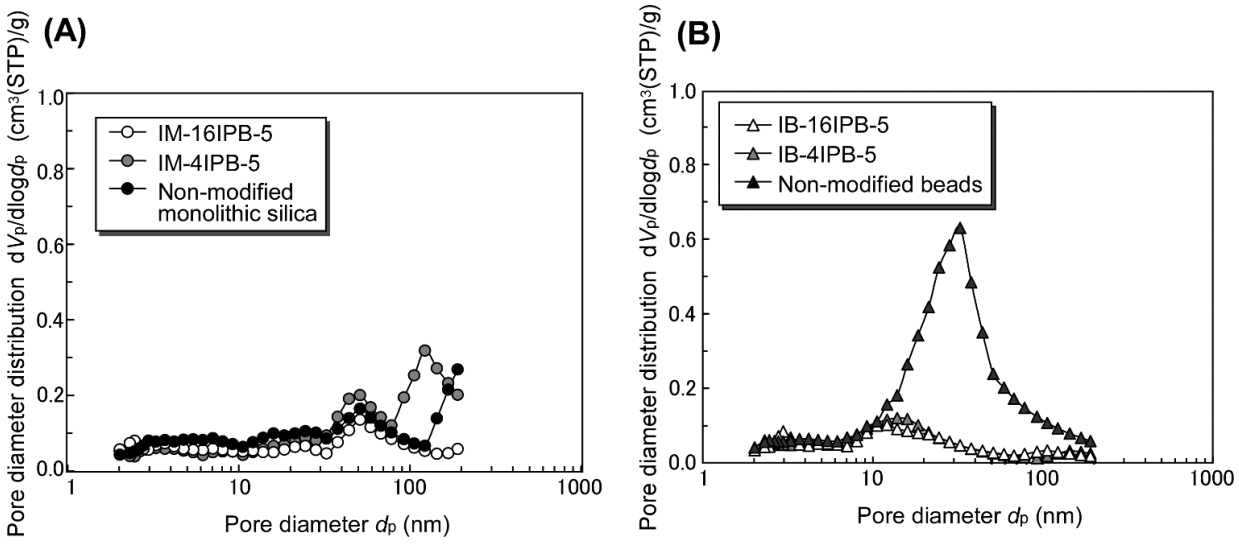


Figure S6. Pore diameter distributions of the prepared monolithic silica rod (A) and silica beads (B). The silica matrix abbreviations are defined in Table 2, and the obtained surface areas are given in Table 3.

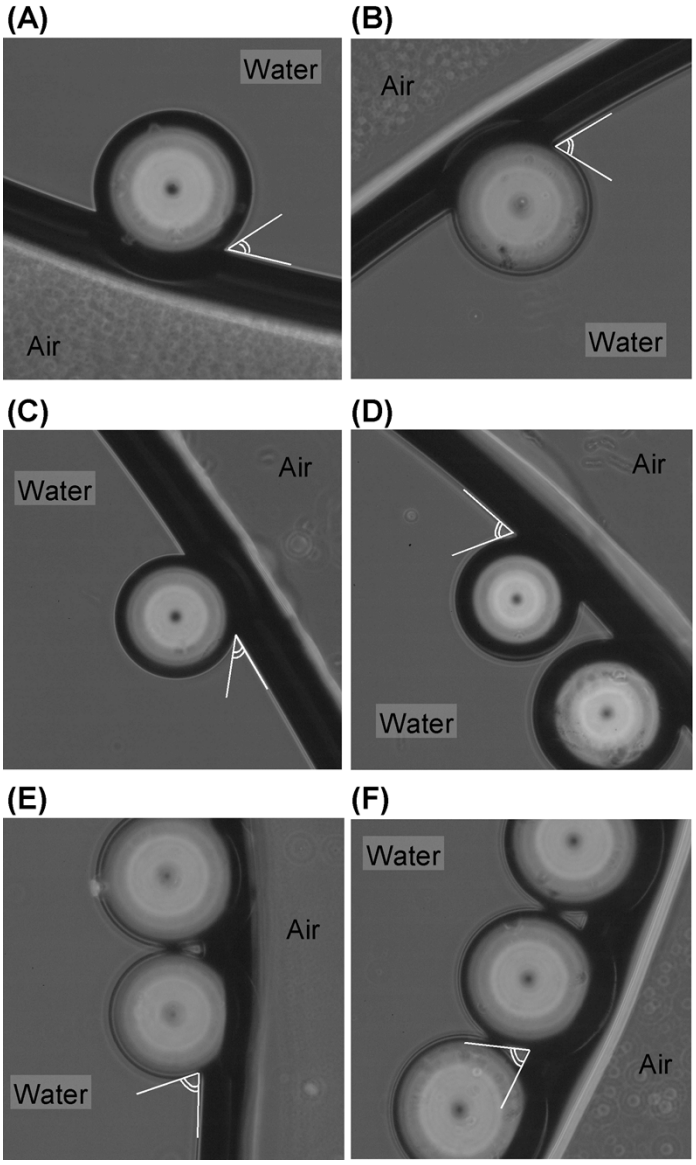


Figure S7. Microscopic observations of ATRP-initiator-modified and P(IPAAm-*co*-BMA)-grafted glass beads at water–air interface between two plane parallel microscope slides. Microphotographs A and B show short P(IPAAm-*co*-BMA)-grafted glass beads in water at 10 and 50 °C, respectively. C and D show long P(IPAAm-*co*-BMA)-grafted glass beads in water at 10 and 50 °C, respectively. E and F show ATRP-initiator-modified glass beads in water at 10 and 50 °C, respectively. The dark line is an optical effect caused by the curved liquid–air interface.

Table S4. Nitrogen microanalysis of P(IPAAm-*co*-BMA) brush grafted glass beads.

Sample	ATRP Reaction time (h)	Nitrogen composition (μg/g)	Grafted copolymer (mg/m2)
Short P(IPAAm-*co*-BMA) brush-grafted glass beads	4	5.16 ± 0.06	4.22
Long P(IPAAm-*co*-BMA) brush-grafted glass beads	16	6.35 ± 0.12	5.20

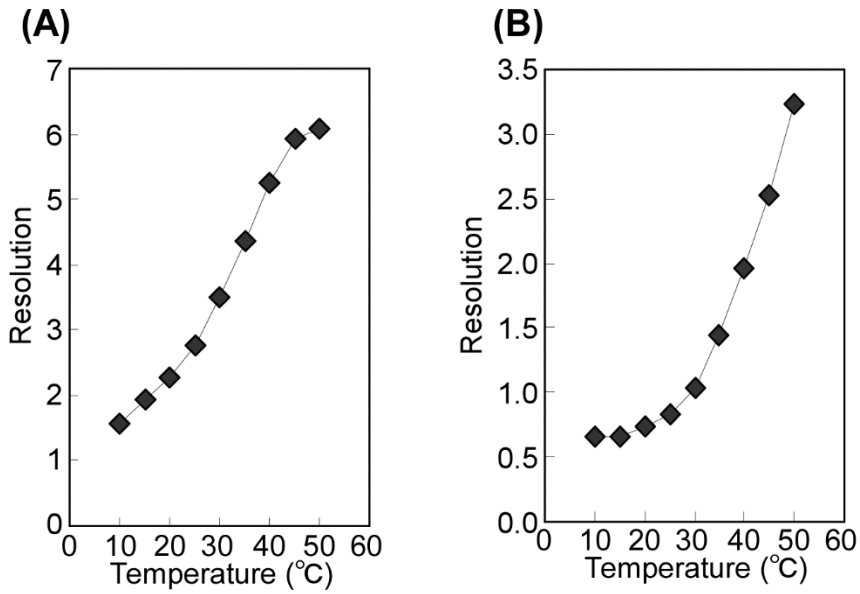


Figure S8. Resolution between methyl benzoate and ethyl benzoate on (A) long P(IPAAm-*co*-BMA)-brush-grafted monolithic silica rod columns (IM-16IPB-5 in Table 2), and (B) long P(IPAAm-*co*-BMA)-brush-grafted silica bead columns (IB-16IPB-5).

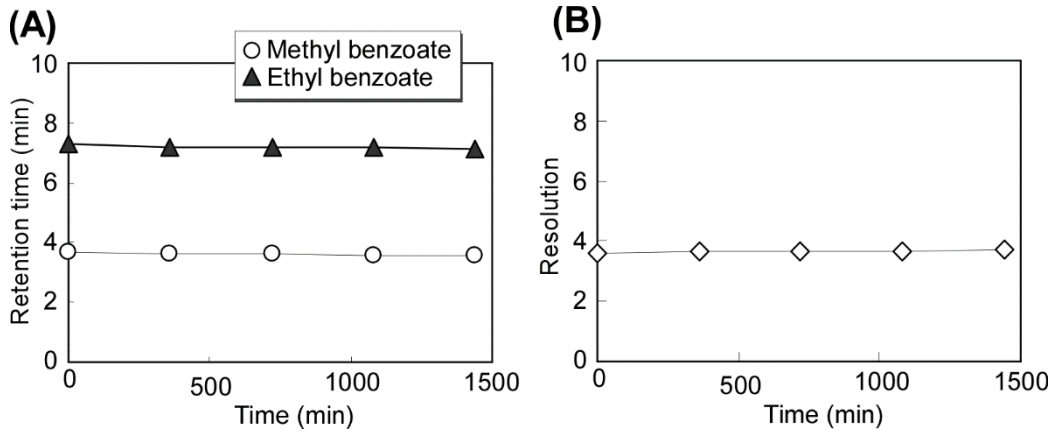


Figure S9. Time-course of (A) retention time of methyl benzoate and ethyl benzoate and (B) resolution on long P(IPAAm-*co*-BMA)-brush-grafted monolithic silica rod columns (IM-16IPB-5 in Table 2).

Table S5. Back pressure before and after continuous flowing of mobile phase.

Time (h)	Back pressure (kg/cm^2) [a)]
0	35
48	31

a) Measured with flowing Milli-Q water as the mobile phase at a flow rate of 1 mL/min at 30 °C.

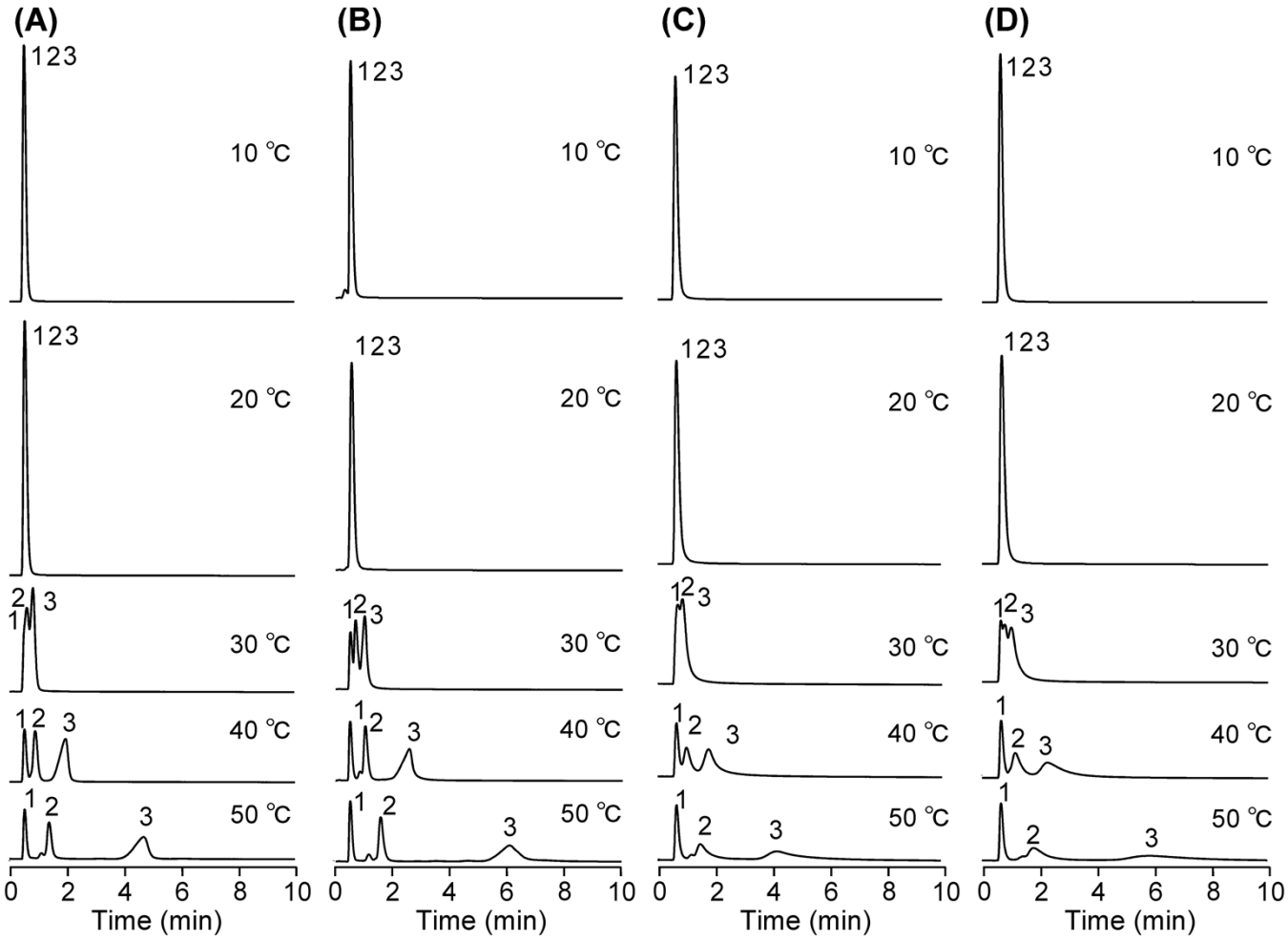


Figure S10. Elution profiles of insulin fragments from poly(IPAAm-*co*-BMA)-brush-grafted monolithic silica and silica bead packing materials. (A) long and (B) short P(IPAAm-*co*-BMA)-brush-grafted monolithic silica rod columns (IM-16IPB-5 and IM-4IPB-5 in Table 2), and (C) long and (D) short P(IPAAm-*co*-BMA)-brush-grafted silica bead columns (IB-16IPB-5 and IB-16IPB-5 in Table 2). The mobile phase was 66.7 mM phosphate buffer (pH 7.0). Peak Nos.1, 2, and 3 represent insulin chain A, insulin chain B, and insulin, respectively.

(S1) Hansch, C.; Albert, L.; Hoekman, D., *In Exploring QSAR: Hydrophobic, Electronic and Steric Constant* American Chemical Society: Washington DC, **1995**; p.174, 178.