Supporting Information for:

Synthesis of a novel carboxybetaine copolymer with different spacer lengths and inhibition of nonspecific protein adsorption on its polymer film

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The supporting information are composed of 9 figures and 1 table in 11 pages

List of abbreviations

AIBN: 2,2'-Azobis(isobutyronitrile)

anti-HSA: Antihuman serum albumin

DMAEMA/PDMAEMA: *N,N*-Dimethylaminoethyl methacrylate/ poly(*N,N*-dimethylaminoethyl methacrylate)

EDC: 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride

FBS: Fetal bovine serum

NHS: *N*-Hydroxysuccinimide

NMR: Nuclear magnetic resonance

RAFT: Reversible addition-fragmentation chain-transfer

SEC: Size exclusion chromatography

SPR: Surface plasmon resonance

TFA: Trifluoroacetic acid

XRR: X-ray reflectometry
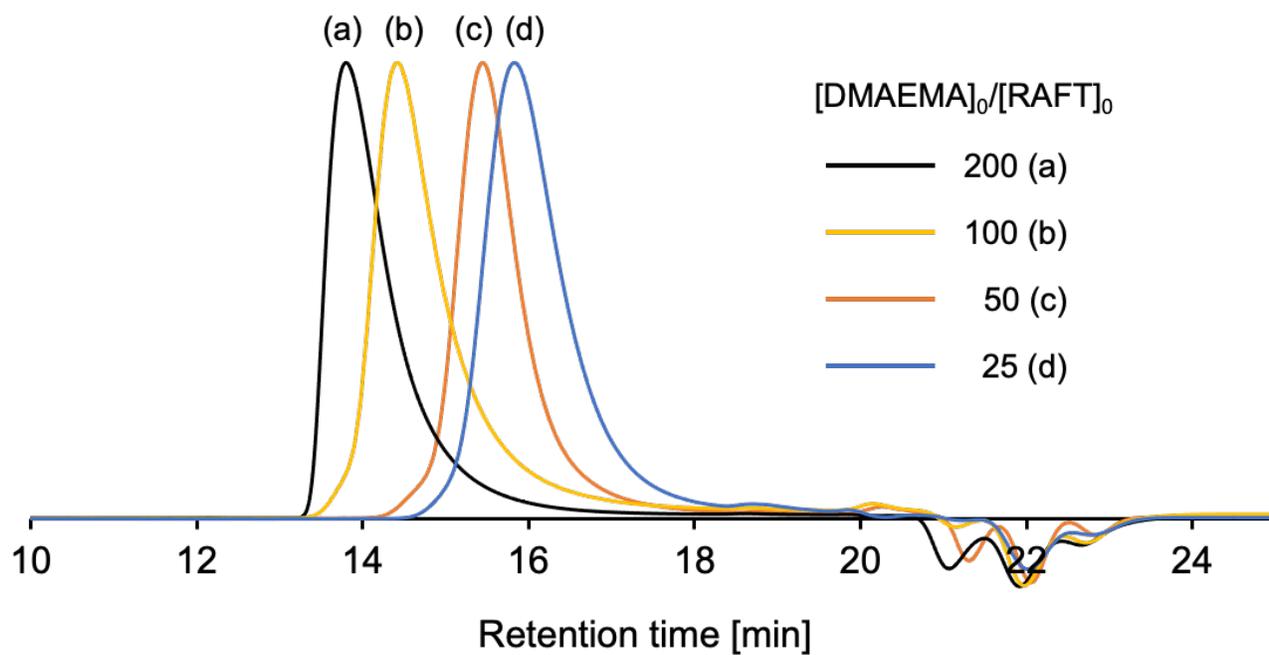
Figure S1. SEC curves of PDMAEMA obtained with AIBN / RAFT agent initiating system in toluene at 60 °C. $[DMAEMA]_0 = 500$ mM. $[DMAEMA]_0/[RAFT\ agent]_0 = 200$ (a), 100 (b), 50 (c), 25 (d). $[AIBN]_0/[RAFT\ agent]_0 = 0.3$.
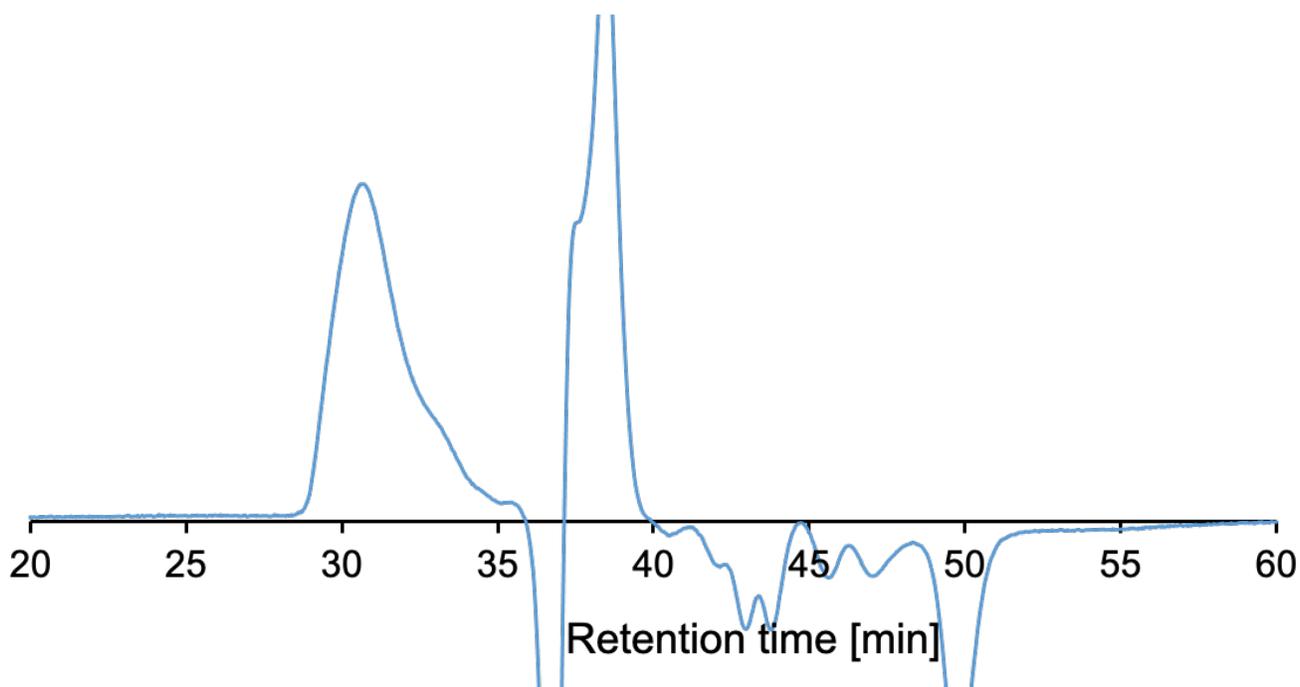
Figure S2. SEC curves of P(CBMA2) obtained with VA-044 / RAFT agent initiating system in acetate buffer (pH 5.2) at 37 $^\circ$ C. $[CBMA2]_0$ = 500 mM. $[CBMA2]_0/[RAFT\ agent]_0$ = 200. $[VA\text{-}044]_0/[RAFT\ agent]_0$ = 0.3.

Table S1. CBMA1/CBMA3 composition and unreacted DMAEMA unit ratio of P(CBMA1/CBMA3) and amount of coated P(CBMA1/CBMA3) on the surface of SPR sensor chips.

PDMAEMA (Theoretical chain length)	PCBMA1/PCBMA3		Ratio of unreacted DMAEMA (%) [b]	Amount of coated P(CBMA1/CBMA3) [c] (ng cm^{-2})	Thickness of the coated P(CBMA1/CBMA3)[d] (nm)
	Theoretical composition	Measured composition by ^{1}H NMR [a]			
25 mer	100:0	100:0	1	332 ± 6	
	80:20	83:17	4	296 ± 16	
	0:100	0:100	7	319 ± 19	
50 mer	100:0	100:0	4	313 ± 18	
	90:10	92:8	4	274 ± 10	
	80:20	79:21	9	293 ± 12	
	60:40	66:34	10	277 ± 3	
	0:100	0:100	30	305 ± 9	
100 mer	100:0	100:0	2	328 ± 25	
	80:20	80:20	6	311 ± 9	
	0:100	0:100	25	353 ± 29	
200 mer	100:0	100:0	1	368 ± 6	3.0
	80:20	77:23	5	357 ± 11	3.5
	60:40	63:37	8	249 ± 24	2.7
	0:100	0:100	29	392 ± 18	2.9

a: Polymer composition was calculated using peak integration ratio of methyl protons on quaternary ammonium of CBMA1 (3.30~3.35 ppm) and CBMA3 (3.20~3.25 ppm) by ^{1}H NMR.

b: The unreacted unit ratio was calculated using peak integration ratio of methyl protons on quaternary ammonium of protonated DMAEMA (~3.0 ppm) unit as shown Figure S1(C).

c: Amount of coated P(CBMA1/CBMA3) was measured by SPR.

d: Measured by X-ray reflectometry (XRR, Malvern Panalytical, X'Pert PRO-MRD), X-ray: Cu Kα, λ = 1.54 Å. The surface roughness of the SPR chips was evaluated by AFM before use based on ISO25178. The arithmetic mean height of the surface (Sa) and the root mean square height of the surface (Sq) of two different chips were 0.652 and 0.630 nm for Sa and 0.807 and 0.788 nm for Sq, respectively.
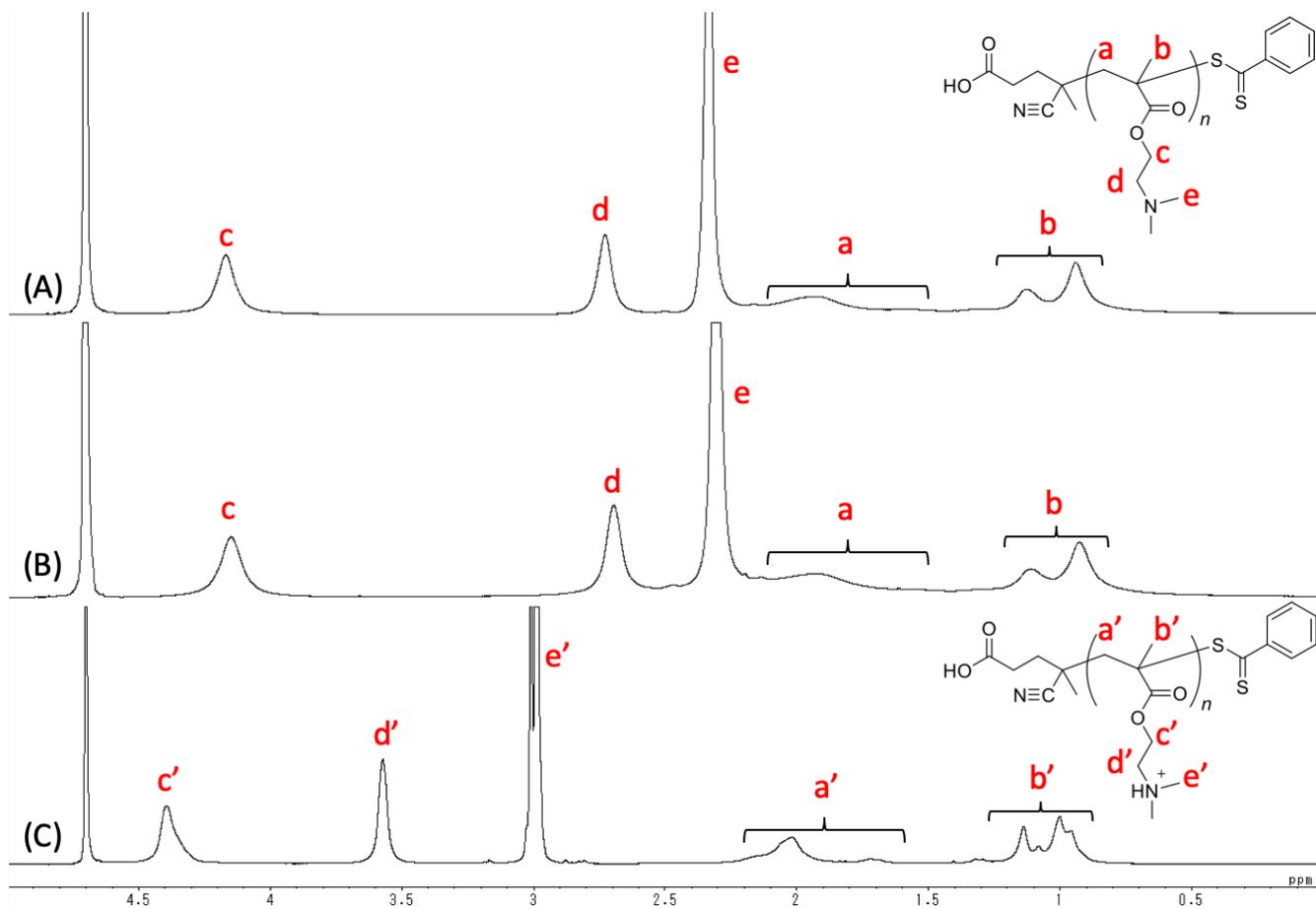
Figure S3. ^{1}H NMR spectra of PDMAEMA in D$_2$O (A), 100 mM NaOD solution (B), 100 mM DCl solution (C).
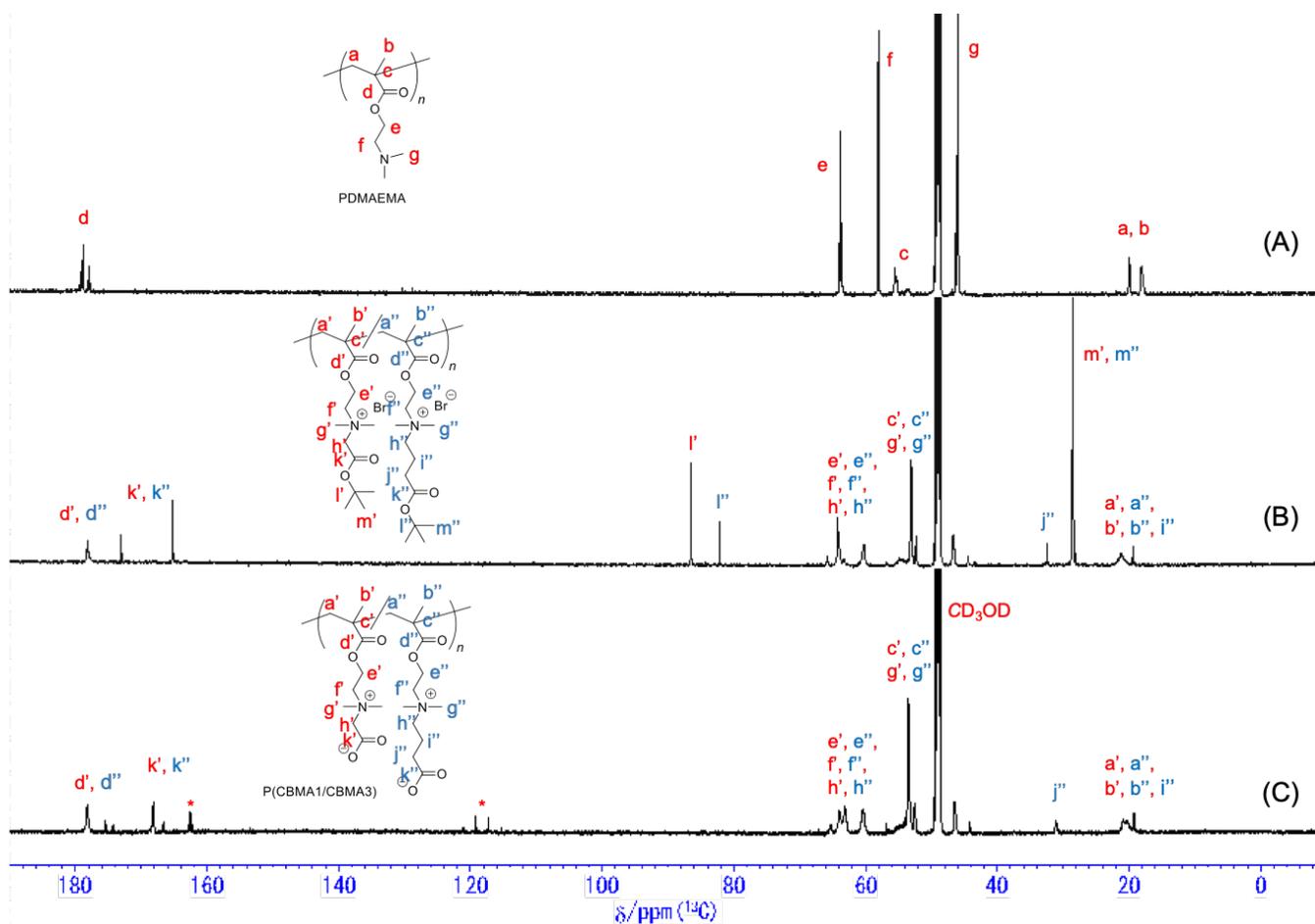
Figure S4. ^{13}C NMR spectra of PDMAEMA (A), quarternized PDMAEMA (B), and P(CBMA1/CBMA3) (C) in CD$_3$OD. Signals (a) at 162 ppm and ca. 120 ppm are assigned as remaining TFA, which was removed by dialysis before coating on SPR sensor chips and following measurements.
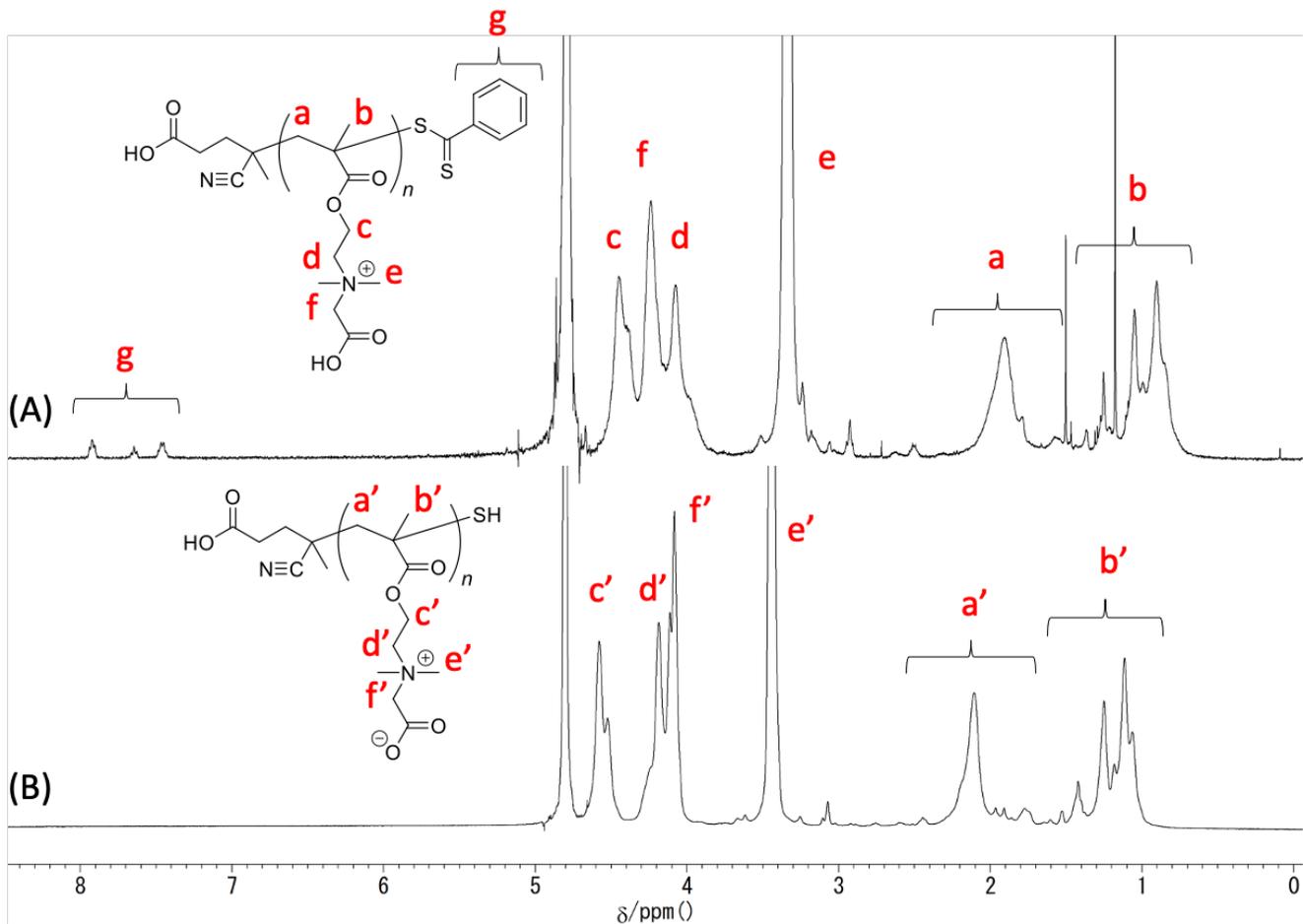
Figure S5. ^{1}H NMR spectra of PCBMA1 before (A) and after (B) RAFT group removal by TCEP/ethanolamine reduction.
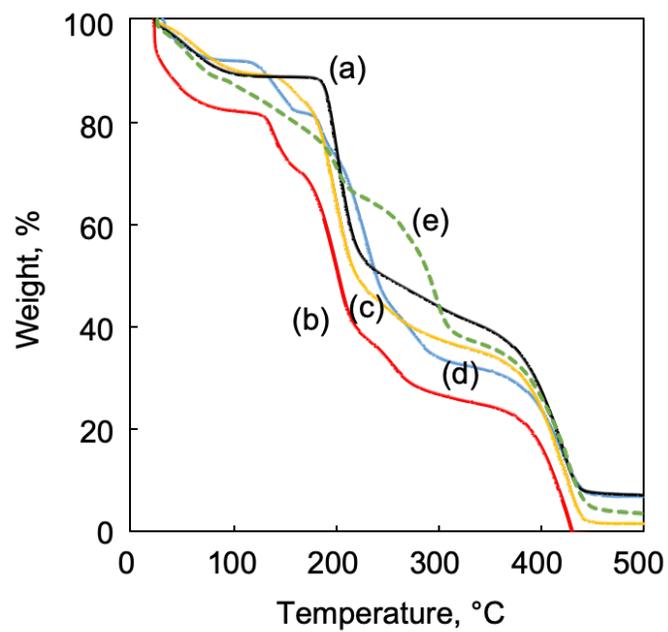
Figure S6. Overall view of thermo-gravimetric analyses of carboxybetaine (co)polymers with different spacer lengths. (a) PCBMA1, (b) P(CBMA1/CBMA3) (80/20), (c) P(CBMA1/CBMA3) (60/40), (d) PCBMA3, and (e) PCBMA2 (control).
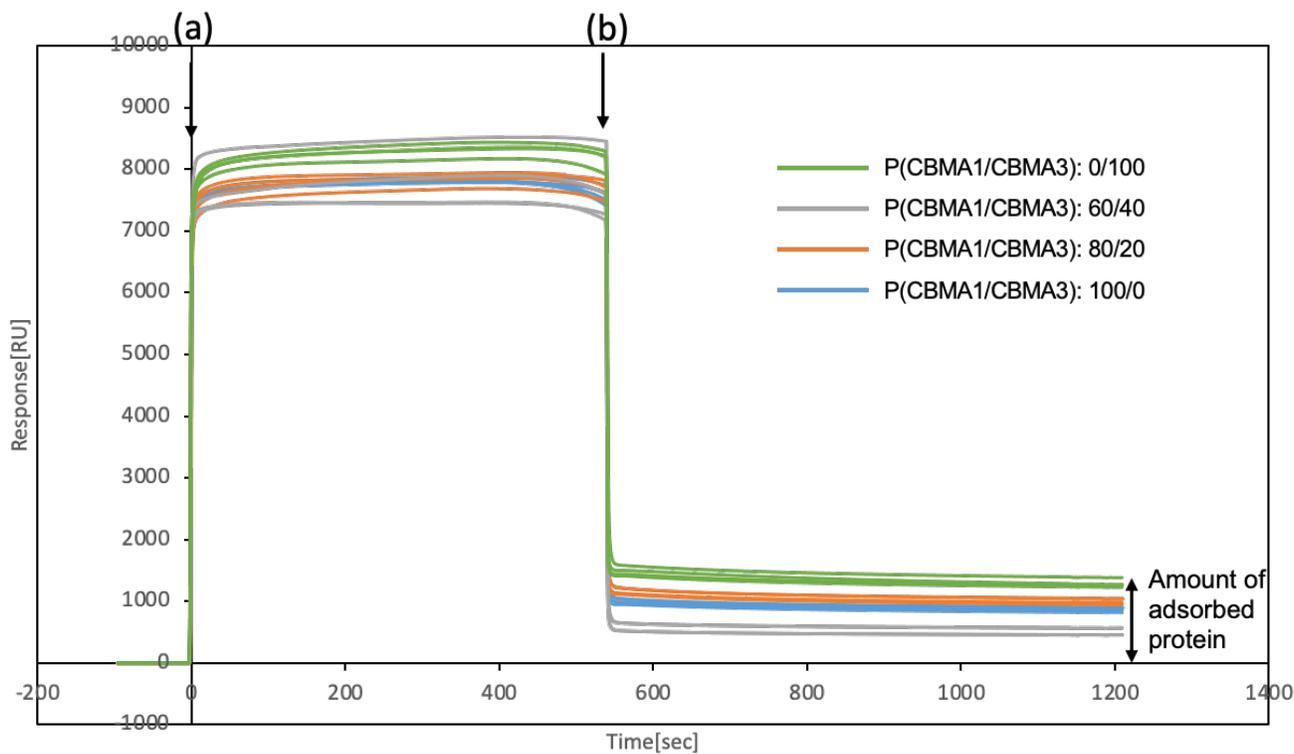
Figure S7. Nonspecific protein adsorption on P(CBMA1/CBMA3) (50 mer)-coated surface characterized by SPR. P(CBMA1/CBMA3) = 0/100 (green), 60/40 (gray), 80/20 (orange), 100/0 (blue). Procedure: (a) injection of FBS for 540 sec, (b) end of injection. SPR response of 1000 RU = 100 ng/cm^2. Lines of same color are results of different sensor chips coated with the same P(CBMA1/CBMA3) components.
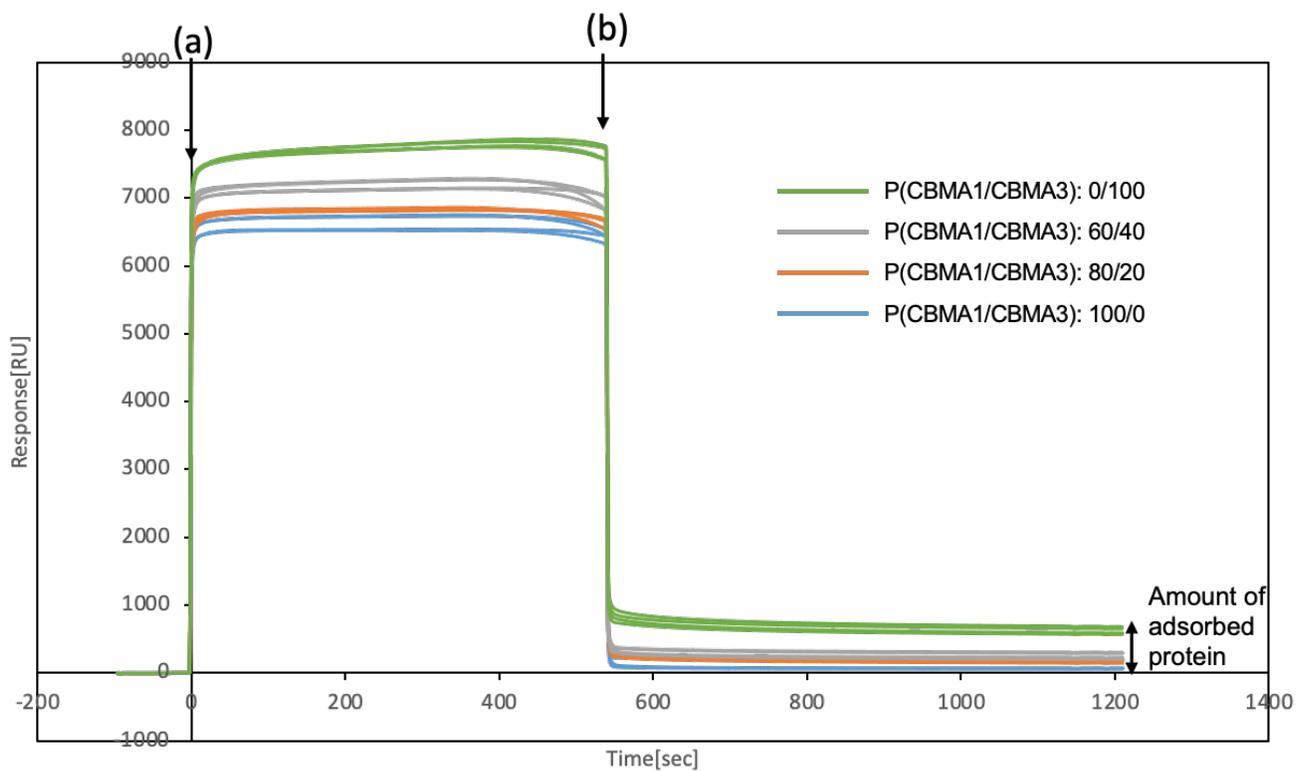
Figure S8. Nonspecific protein adsorption on P(CBMA1/CBMA3) (200 mer)-coated surface characterized by SPR. P(CBMA1/CBMA3) = 0/100 (green), 60/40 (gray), 80/20 (orange), 100/0 (blue). Procedure: (a) injection of FBS for 540 sec, (b) end of injection. SPR response of 1000 RU = 100 ng/cm^2. Lines of same color are results of different sensor chips coated with the same P(CBMA1/CBMA3) components.
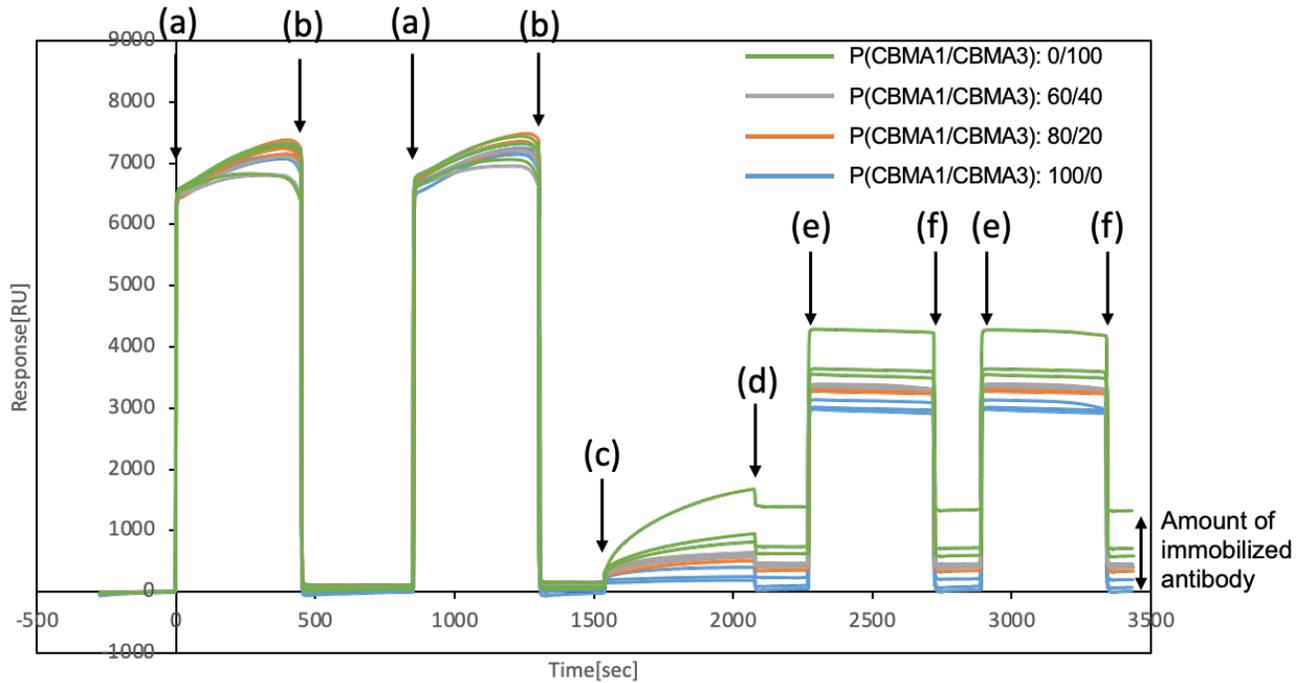
Figure S9. Antibody immobilization on P(CBMA1/CBMA3)-coated surface via NHS/EDC activation characterized by SPR. P(CBMA1/CBMA3) = 0/100 (green), 60/40 (gray), 80/20 (orange), 100/0 (blue). Procedure: (a) injection of NHS/EDC (0.05 M/0.2 M) solution for 450 sec, (b) end of injection of NHS/EDC, (c) anti-HSA antibody (0.1 mg/mL) for 540 sec, (d) end of injection of anti-HSA antibody, (e) injection of 10mM carbonate buffer (pH9.0) with 0.3 M NaCl, (f) end of injection of carbonate buffer. SPR response of 1000 RU = 100 ng/cm^2. Lines of same color are results of different sensor chips coated with the same P(CBMA1/CBMA3) components.