frontiers

Supplementary Material

Modulation of Viscoelasticity and Interfacial Potential of Polyelectrolyte Brush by Ion-Specific Interactions

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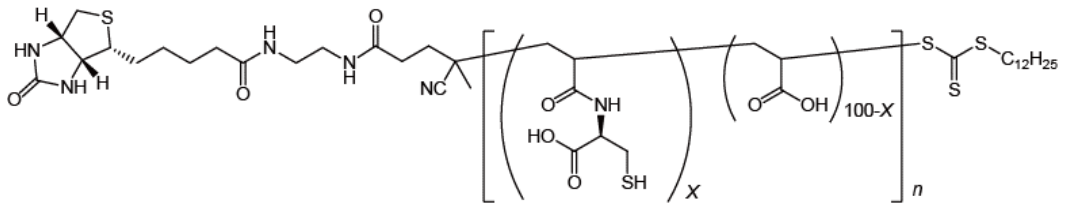
Supplementary Material S1. Synthesis and basic characterization of polymers used in this study

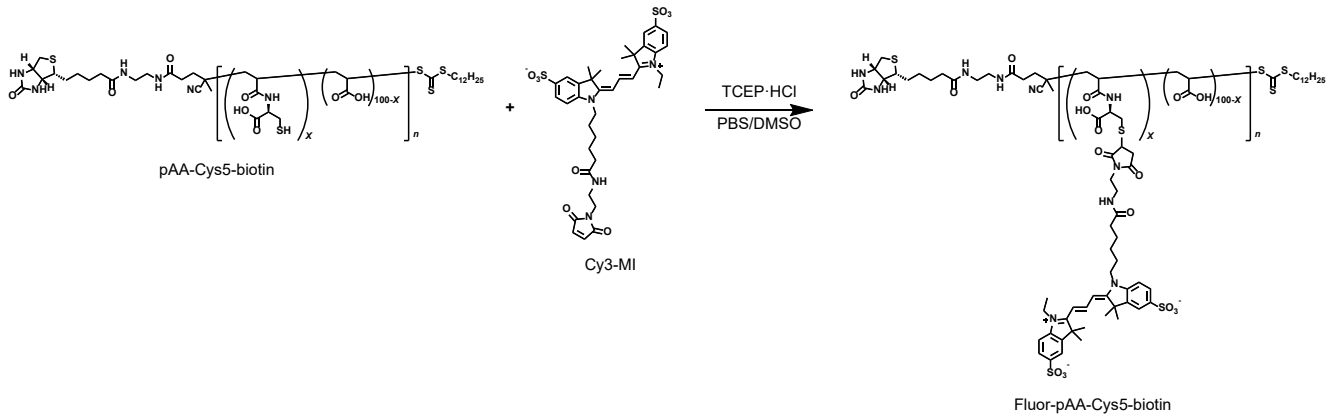
S1a. pAA-Cys*X*-biotin



Supplementary Figure S1a. pAA-Cys*X*-biotin

pAA-Cys*X*-biotin was synthesized through copolymerization of *S*-trityl-cysteine acrylamide (*S*-Tri-Cys-AAm) and acrylic acid (AA) using 4,4'-((*E*)-diazene-1,2-diyl)bis(4-cyano-*N*-(2-(5-((3a*R*,4*R*,6a*S*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamido)ethyl)pentanamide) (ACVA-biotin) as an initiator and 2-(dodecylthiocarbonothioylthio)-2-methylpropionic acid (DDMAT) as a chain transfer agent, followed by deprotection of trityl group with trifluoroacetic acid (TFA). Briefly, *S*-Tri-Cys-AAm (0.01*X* mmol), AA (1-0.01*X* mmol), ACVA-biotin (0.01 mmol), and DDMAT (0.01 mmol) were dissolved in 1 mL of dimethylsulfoxide (DMSO) dried with molecular sieves 4A. The solution was purged with nitrogen gas for 1 h, sealed, and heated in an oil bath at 70 °C overnight. After cooling down to room temperature, the solution was poured into acetone (10 mL) with stirring. The resultant oily precipitate was collected with centrifugation (3,500 rpm, 5 min.). After removing supernatant by decantation, trifluoroacetic acid (TFA) (1 mL) was added and stirred for 1 h at r.t. The solution was poured into diethyl ether (10 mL). The resultant precipitate was washed with diethyl ether (10 mL) three times and dried in vacuum at r.t. Successful polymerization and deprotection were confirmed by ^{1}H NMR spectra recorded at 400 MHz with a JNM–ECS400 NMR spectrometer (JEOL, Tokyo, Japan). The weight average molecular weight and polydispersity index (M_w, M_w/M_n) of pAA-Cys0-biotin, pAA-Cys5-biotin, and pAA-Cys20-biotin were estimated to be (1.7×10^4, 1.1), (1.7×10^4, 2.3), and (2.8×10^4, 1.6), respectively.

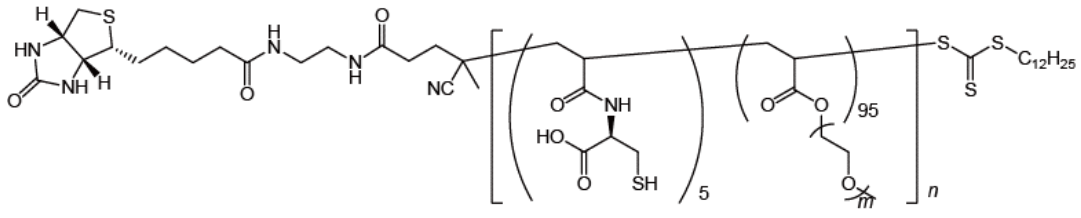
S1b. Fluor-pAA-Cys5-biotin



Supplementary Figure S1b. Fluor-pAA-Cys5-biotin

pAA-Cys5-biotin (50 mg) and tris(2-carboxyethyl)phosphine hydrochloride (TCEP-HCl) (15 mg) were dissolved in phosphate-buffered saline (PBS) (5 mL) under nitrogen atmosphere. pH was adjusted at 7.4 by addition of 1 M NaOH aq. (500 µL). A 1 mg/mL solution of Cy3-maleimide (Cy3-MI) in DMSO (7.2 µL) was then added and stirred overnight at r.t. The solution was transferred in a dialysis tube (molecular weight cut off = 1 kDa) and dialyzed against Milli-Q water (500 mL) for three days with exchanging water once per day. The aqueous solution remained in the tube was then freeze-dried for two days to obtain of Fluor-pAA-Cys5-biotin as a pink powder.

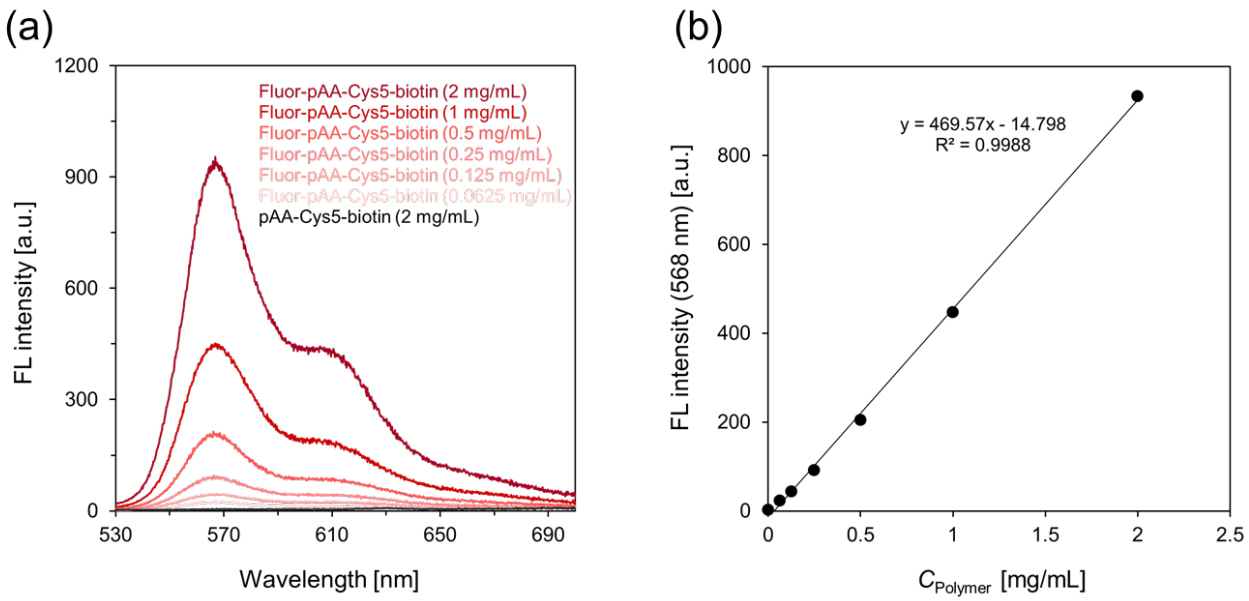
S1c. PEGMA-Cys5-biotin



Supplementary Figure S1c. pPEGMA-Cys5-biotin

pPEGMA-Cys5-biotin was synthesized through copolymerization of *S*-trityl-cysteine acrylamide (*S*-Tri-Cys-AAm) and poly(ethylene glycol) methyl ether acrylate (average M_n 480) using ACVA-biotin as an initiator and DDMAT as a chain transfer agent, followed by deprotection of trityl group with TFA. Briefly, *S*-Tri-Cys-AAm (0.05 mmol), AA (0.95 mmol), ACVA-biotin (0.01 mmol), and DDMAT (0.01 mmol) were dissolved in 1 mL of dimethylsulfoxide (DMSO) dried with molecular sieves 4A. The solution was purged with nitrogen gas for 1 h, sealed, and heated in an oil bath at 70 °C overnight. After cooling down to room temperature, the solution was poured into acetone/hexane (1/1, v/v) (10 mL) with stirring. The resultant oily precipitate was collected with centrifugation (3,500 rpm, 5 min.). After removing supernatant by decantation, trifluoroacetic acid (TFA) (1 mL) was added and stirred for 1 h at r.t. The solution was poured into diethyl ether/hexane (1/1, v/v) (10 mL). The resultant oily precipitate was washed with diethyl ether (10 mL) three times and dried in vacuum at r.t. Successful polymerization and deprotection were confirmed by ^{1}H NMR spectra. GPC analysis of pPEGMA-Cys5-biotin indicated the weight average molecular weight and polydispersity index (M_w, M_w/M_n) of pPEGMA-Cys5-biotin to be (2.1×10^4, 1.6).

S1d. Fluorescence characterization of Fluor-pAA-Cys5-biotin.



Supplementary Figure S1d. (a) Fluorescence spectra for Fluor-pAA-Cys5-biotin (0.0625, 0.125, 0.25, 0.5, 1, and 2 mg/mL) and pAA-Cys5-biotin (2 mg/mL) in Tris-HCl buffer (10 mM, pH 7.4, with 100 mM NaCl). λ_{ex} = 520 nm. (b) Fluorescence intensity at λ = 568 nm plotted vs. concentration of Fluor-pAA-Cys5-biotin ($C_{Polymer}$).

Supplementary Material S2. Fitting of supported membrane and neutravidin layer.

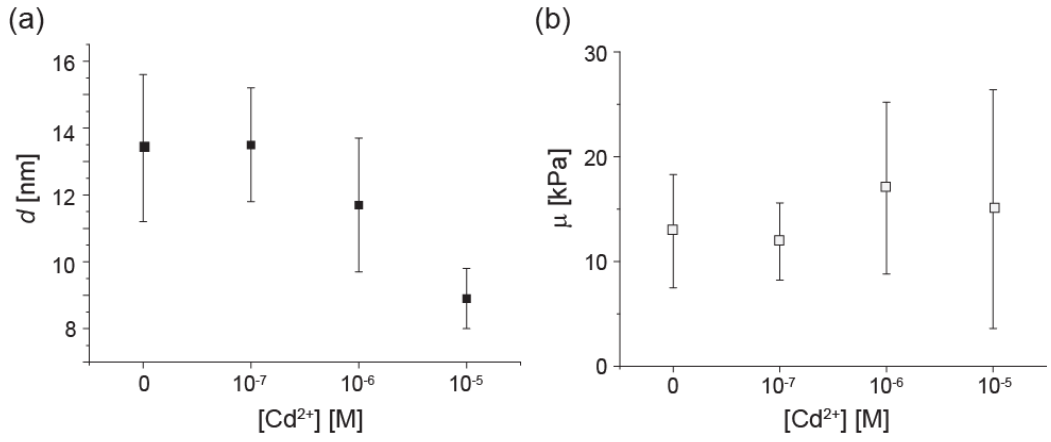
	Thickness d [nm]	Shear elasticity μ [kPa]	Viscosity η [mPa·s]
Supported membrane	6.1 ± 0.1	143.0 ± 14.2	2.1 ± 0.0
Neutravidin	8.7 ± 0.0	1050	0.7 ± 0.1

Supplementary Table S2. Summary of the layer parameters calculated from QCM-D data presented in Figure 3a, calculated with Voigt-Voinova model (Vogt et al., *The Journal of Physical Chemistry B,* 108**,** 12685-12690 (2004)). Shear elasticity of neutravidin (1050 kPa) was taken from the previous account (Amadei et al., *Langmuir,* 34**,** 14046-14057 (2018)) and used as a constraint.

Supplementary Material S3. The layer parameters of pAA-Cys5 brushes as a function of $[Cd^{2+}]$.

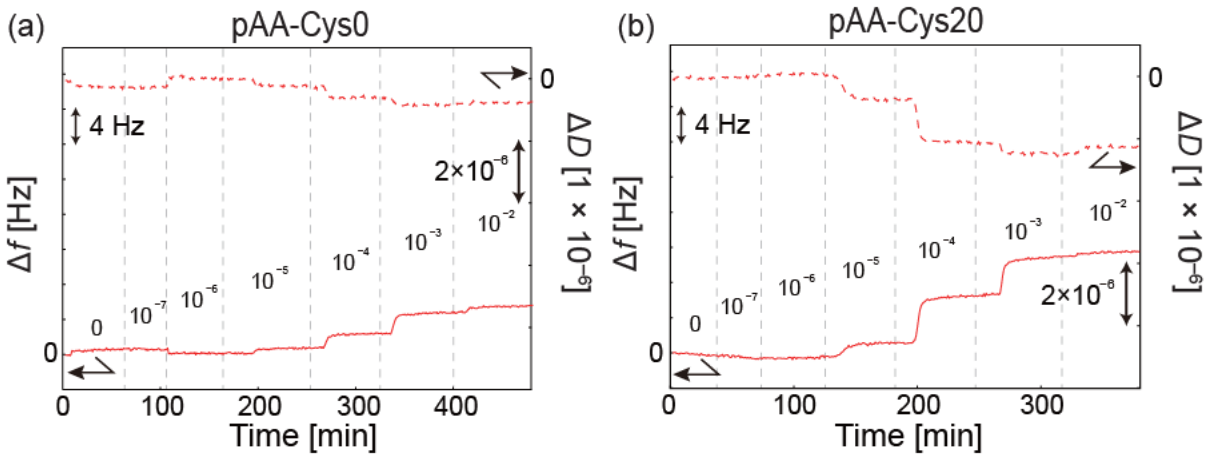
$[Cd^{2+}]$ [M]	Thickness d [nm]	Shear elasticity μ [kPa]	Viscosity η [mPa·s]
0	13.4 ± 2.2	12.9 ± 5.4	1.0 ± 0.1
10^{-7}	13.5 ± 1.7	11.9 ± 3.7	1.0 ± 0.1
10^{-6}	11.7 ± 2.0	17.0 ± 8.2	1.0 ± 0.1
10^{-5}	8.9 ± 0.9	15.0 ± 11.4	1.3 ± 0.2
10^{-4}	4.0 ± 0.6	153.5 ± 130.2	3.6 ± 0.1
10^{-3}	3.9 ± 0.8	120.4 ± 113.2	2.9 ± 0.8
10^{-2}	4.2 ± 0.9	81.0 ± 75.6	2.7 ± 0.8

Supplementary Table S3. The layer parameters of pAA-Cys5 brushes calculated from the QCM-D data presented in Figure 4a. The data were fitted with the same model as the one used in Supplementary Material Table S2. The data presented in grey-shaded cells showed large errors, implying that the model is no longer valid.



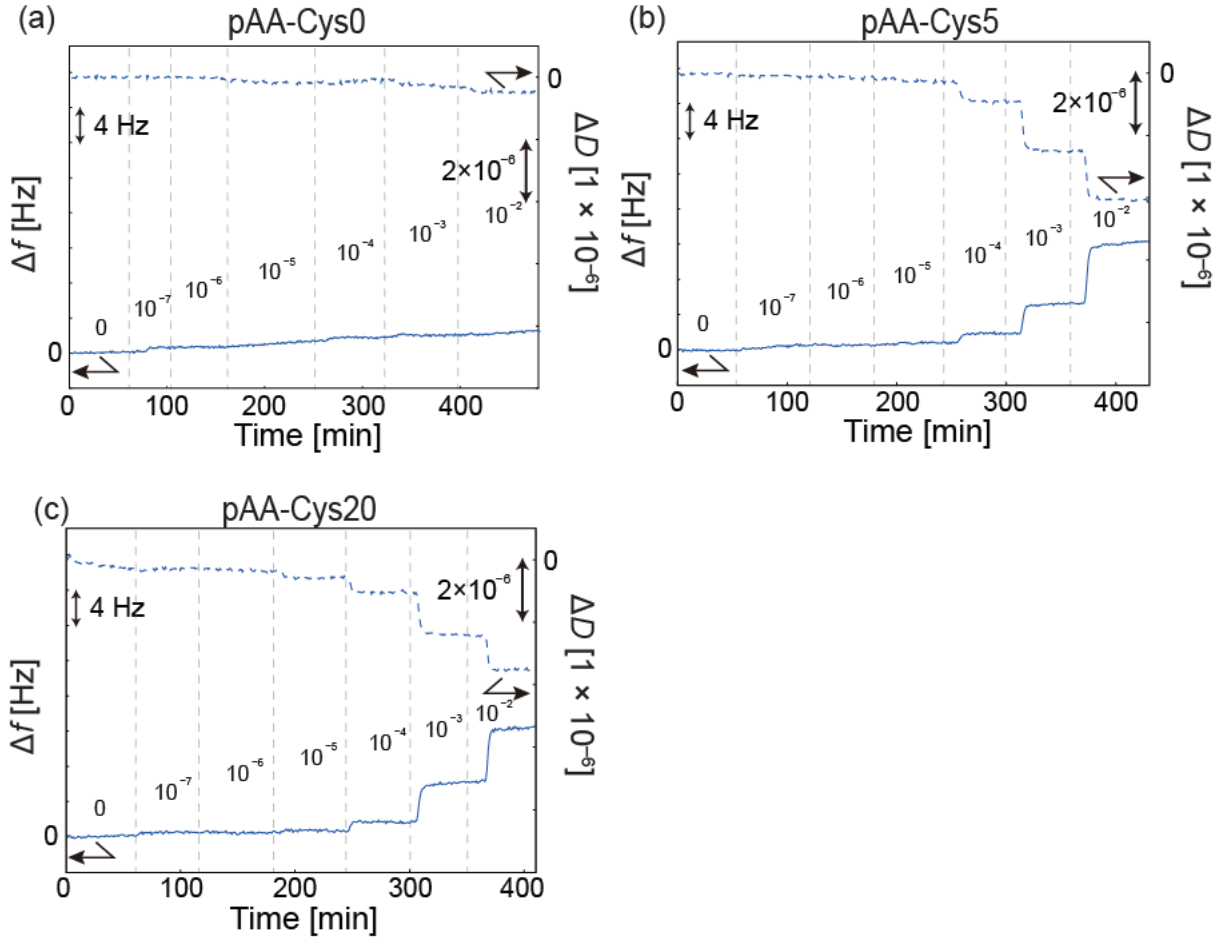
Supplementary Figure S3. Thickness d and shear elasticity η plotted vs. $[Cd^{2+}]$ up to 10^{-5} M.

Supplementary Material S4. QCM-D response of pAA-Cys0 and pAA-Cys20 to Cd^{2+} over time.



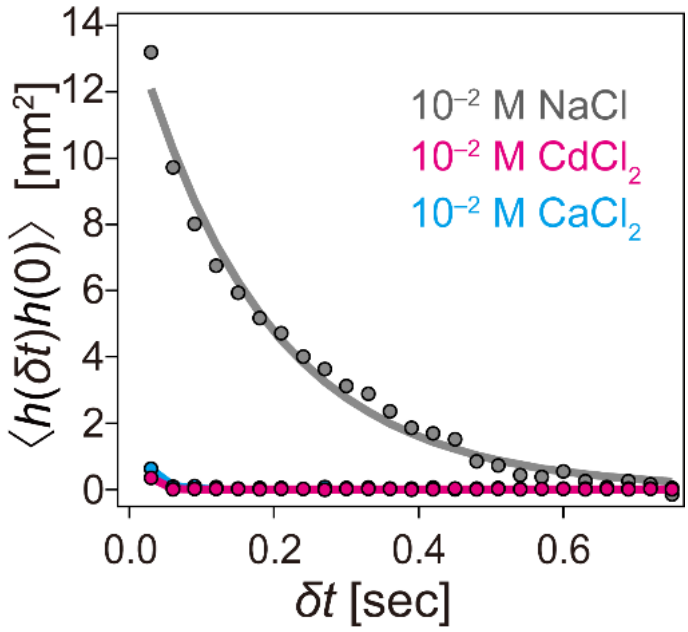
Supplementary Figure S4. Changes in Δf and ΔD of (a) pAA-Cys0 and (b) pAA-Cys20 in response to Cd^{2+} ions.

Supplementary Material S5. QCM-D response of pAA-Cys0, pAA-Cys5 and pAA-Cys20 to Ca^{2+} over time.



Supplementary Figure S5. Changes in Δf and ΔD of (a) pAA-Cys0, (b) pAA-Cys5, and (c) pAA-Cys20 in response to Ca^{2+} ions.

Supplementary Material S6. Autocorrelation functions of latex beads hovering on pAA-Cys5 brushes, measured in the absence of supporting salt (100 mM NaCl)



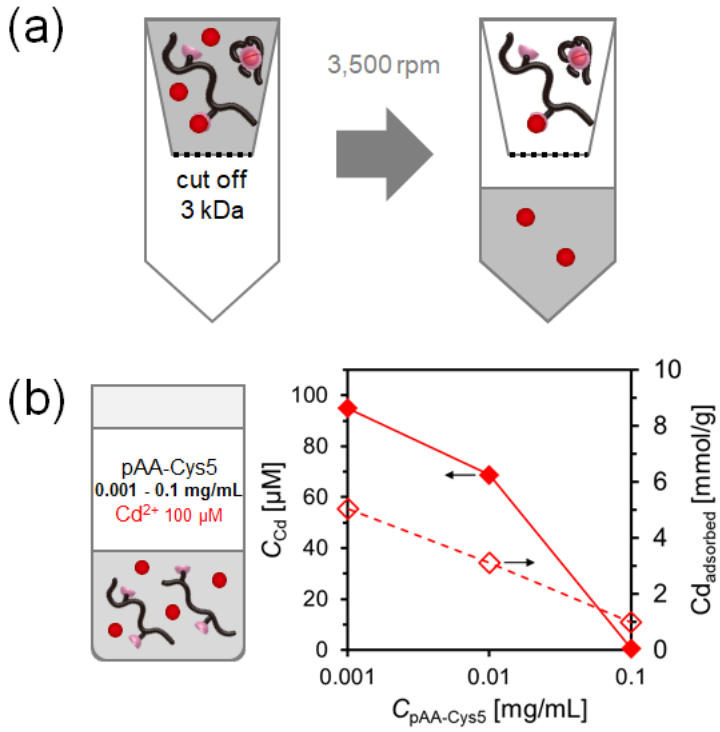
Supplementary Figure S6. Autocorrelation functions of latex beads undergoing vertical Brownian motion on pAA-Cys5 brushes. Note that the electrolytes used in this series of experiments are 10 mM Tris-HCl (pH 7.4) buffer with additional 10 mM NaCl, 10 mM $CdCl_2$, or 10 mM $CaCl_2$.

Supplementary Material S7. Grafting of pAA-Cys5 brushes on particle-supported lipid membranes.

Silica particles (Akzo Nobel, Amsterdam, Netherland) were washed three times with 10 mM Tris-HCl buffer, and the particles were incubated with vesicle suspension (4 mg/mL) in an overhead rotator for 1 h at room temperature. The sample was washed eight times with 1 mL Tris-HCl buffer to remove free vesicles in supernatants. The membrane-coated beads were incubated with neutravidin solution (5 μg/ml) for 1 h. After intensive rinsing of free neutravidin molecules, the sample was incubated with an aqueous solution of the biotinylated polymer (0.1 mg/ml) for overnight. Finally, the unbound polymers were washed off and subjected to the zeta potential measurements.

Supplementary Material S8. Determination of the amount of Cd^{2+} bound to pAA-Cys5.

First, we determined the amount of –SH and –COOH groups per 1 g of polymers, which are 13 mmol/g for –COOH and 0.65 mmol/g for –SH, respectively. Second, as shown in Figure S8a, we varied the concentration of pAA-Cys5 polymers from 0.001 to 0.1 mg/mL while keeping the Cd^{2+} ion concentration constant at 100 µM. The ions that were not captured by pAA-Cys5 pass the ultrafiltration membrane with the cut-off molecular weght of 3 kDa, while the ion-polymer complexes do not because the molecular weight of the polymer is 1.7×10^4 Da (Supplementary Information S1). The Cd^{2+} ion concentration in filtrate was measured by inductively coupled plasma optical emission spectrometry (ICP-OES, SPS7800, Hitachi High-Tech, Tokyo, Japan). This enables one to calculate the amount of captured ions and free ions per unit volume (1 mL). Knowing the polymer concentrations, we could calculate the amount of Cd^{2+} ions bound to 1 g of polymer. In Figure S8b, we plotted the Cd^{2+} ion concentration in filtrate and the amount of Cd^{2+} ions bound to 1 g of polymer as a function of polymer concentration.



Supplementary Figure S8. (a) Schematic illustration of ultrafiltration experiments. (b) Concentration of Cd^{2+} ions in filtrate and the amount of Cd^{2+} ions bound to pAA-Cys5 plotted as a function of polymer concentration.