Electronic Supplementary Material (ESI) for Chemical Communications.

Electronic Supplementary Information

Affinity-mediated capture and release of amphiphilic copolymers for controlling antimicrobial activity

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ESI 1. Experimental procedures, synthetic scheme, and ^{1}H NMR charts.

Materials. Dansyl chloride, triethylamine, methyl methacrylate (MMA), methyl 3-mercaptopropionate (MMP), di-*tert*-butyldicarbonate, methacryloyl chloride were purchased from Acros Organics and used without further purification. 2,2'-azobisisobutyronitrile (AIBN) and the bee venom toxin melittin (purity >85 %) were purchased from Sigma-Aldrich Co. LLC. Methyl-β-cyclodextrin (average MW=1,313) was purchased from Tokyo Chemical Industry Co., Ltd. All other chemicals and solvents were obtained from Sigma-Aldrich Co. LLC. and Thermo Fisher Scientific, Inc. and used without further purification. ^{1}H NMR was performed using a Varian MR400 (400 MHz) and analyzed using VNMRJ 3.2 and MestReNova. Gel permeation chromatography (GPC) analysis was performed using a Waters 1515 HPLC instrument equipped with Waters Styragel (7.8 × 300 mm) HR 0.5, HR 1, and HR 4 columns in sequence and detected by a differential refractometer (RI). *Escherichia coli* ATCC®25922™ was used for model bacteria to evaluate antimicrobial activity of polymers. Human RBCs (leukocytes reduced adenine saline added) were obtained from the American Red Cross Blood Services Southeastern Michigan Region and used prior to the out date indicated on each unit.

Synthesis of dansyl group-conjugated chain transfer agent. A dansyl group-conjugated chain transfer agent was synthesized according to the reported procedure.[1, 2] Briefly, dansyl chloride and cystamine hydrochloride were stirred in DMF in the presence of triethylamine at room temperature overnight. The crude thiol product was purified by silica-gel column chromatography (EtOAc/hexane 1:1). The disuflide product was then mixed with tris(2-carboxyethyl)phosphine in order to reduce the disulfide bond for usage as a chain transfer agent. The crude product was purified by silica-gel column chromatography (EtOAc/ hexane 1:1).

Polymer synthesis. Dye-labeled and non-labeled methacrylate homo- and random copolymers were prepared by previously described procedure with slight modification (Figure S1: Synthesis scheme).[1, 2] Dansyl group-conjugated thiol compound described above was used for dye-labeled polymers, and methyl 3-mercaptopropionate (MMP) was used for non-labeled polymers as a chain transfer agent. The *N*-(*tert*-butoxycarbonyl)aminoethyl methacrylate (Boc-AEMA) and methyl methacrylate (MMA) monomers (0 or 50 mole% of MMA relative to total amount of monomers), AIBN and the chain transfer agent were dissolved in acetonitrile. After the solution was purged with nitrogen, the reaction mixture was heated up to 70 ºC and kept overnight. The

obtained polymers were purified by precipitation into hexane to remove unreacted impurities and then treated by TFA to de-protect Boc-groups. The resulting polymers were precipitated into diethyl ether and collected by centrifugation. Subsequently, they were dissolved in water and lyophilized to give cationic polymers as powders. The Boc-protected polymers were characterized by GPC analysis to measure the number average molecular weight (M_n) and the weight average molecular weight (M_w) calculated using a calibration curve based on 10 standard samples of poly(methyl methacrylate), MW 500–50,000 (Agilent Technologies, M-L-10, no. PL2020-0100). The polymers were also characterized by ^{1}H NMR analysis to determine the degree of polymerization (DP), the mole percentage of MMA (MP_{methyl}), and consecutive the number average molecular weight (M_n) of Boc-protected and de-protected polymers (Figure S2-S5: ^{1}H NMR charts of final de-protected polymers). ^{1}H NMR ($CDCl_3$, 400 MHz) for non-labeled homopolymer (PM_0), protected, MP_{methyl}=0, and DP=12: σ=4.01 (bs, 24.91H), 3.69 (s, 3H), 3.38 (bs, 24.61H), 2.8-2.5 (bm, 7H), 2.3-0.8 (bm, 176.98H). ^{1}H NMR (CD_3OD, 400 MHz) for non-labeled homopolymer (PM_0), de-protected, MP_{methyl}=0, and DP=12: σ=4.21 (bs, 24.41H), 3.66 (s, 3H), 2.8-2.5 (bm, 7H). ^{1}H NMR ($CDCl_3$, 400 MHz) for non-labeled comopolymer (PM_{43}), protected, MP_{methyl}=45, and DP=15: σ=4.03 (bs, 16.04H), 3.69 (s, 3H), 3.59 (bs, 19.34H), 3.39 (bs, 16.11H) 2.9-2.4 (bm, 7H), 2.3-0.8 (bm, 157.23H). ^{1}H NMR (CD_3OD, 400 MHz) for non-labeled copolymer (PM_{43}), de-protected, MP_{methyl}=43, and DP=17: σ=4.26 (bs, 18.96H), 3.71 (s, 3H), 3.66 (bs, 21.05H), 2.8-2.5 (bm, 7H), 2.3-0.8 (bm, 86.89H). ^{1}H NMR ($CDCl_3$, 400 MHz) for dansyl-labeled homopolymer (D-PM_0), protected, MP_{methyl}=0, and DP=10: σ=8.56 (d, 0.77H), 8.25 (m, 1.74H), 7.55 (m, 2.01H), 7.21 (d, 1.13H), 4.02 (bs, 20.11H), 3.38 (bs, 20.64H), 3.04 (bs, 2.29H), 2.89 (s, 6H), 2.8-2.3 (bm, 5.03H), 2.3-0.8 (bm, 159.51H). ^{1}H NMR (CD_3OD, 400 MHz) for dansyl-labeled homopolymer (D-PM_0), de-protected, MP_{methyl}=0, and DP=12: σ=8.56 (d, 0.87H), 8.21 (d, 0.85H), 8.10 (d, 0.96H), 7.55 (t, 1.77H), 7.21 (d, 0.85H), 4.13 (bs, 24.78H), 2.79 (s, 6H), 2.7-2.3 (bm, 4.09H), 2.3-0.8 (bm, 63.68H). ^{1}H NMR ($CDCl_3$, 400 MHz) for dansyl-labeled copolymer (D-PM_{41}), protected, MP_{methyl}=43, and DP=10: σ=8.56 (d, 0.83H), 8.25 (m, 1.82H), 7.56 (m, 2.08H), 7.21 (d, 1.16H), 4.04 (bs, 11.12H), 3.60 (bs, 12.90H), 3.39 (bs, 11.66H), 3.04 (bs, 2.43H), 2.90 (s, 6H), 2.8-2.3 (bm, 5.01H), 2.3-0.8 (bm, 111.28H). ^{1}H NMR (CD_3OD, 400 MHz) for dansyl-labeled copolymer (D-PM_{41}), de-protected, MP_{methyl}=41, and DP=15): σ=8.56 (d, 0.93H), 8.21 (d, 0.89H), 8.10 (bs, 1.03H), 7.55 (t, 1.92H), 7.21 (d, 1.07H), 4.13 (bs, 17.43H), 3,53 (bs, 17.80H), 2.80 (s, 6H), 2.8-2.3 (bm, 4.91H), 2.3-0.8 (bm, 74.27H).

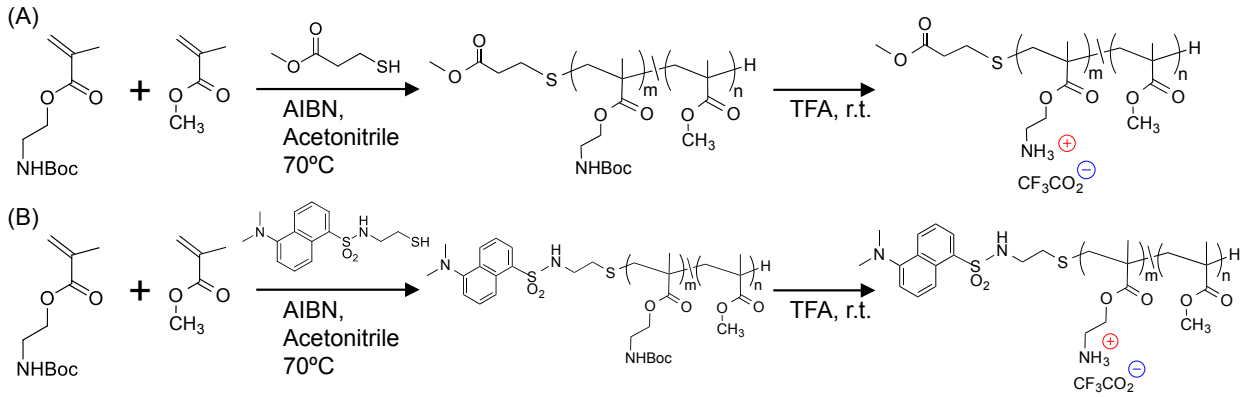

Figure S1. Synthetic scheme of methacrylate random copolymers using methyl-3-mercaptopropionate (A) and dansyl-group modified chain transfer agent (B).

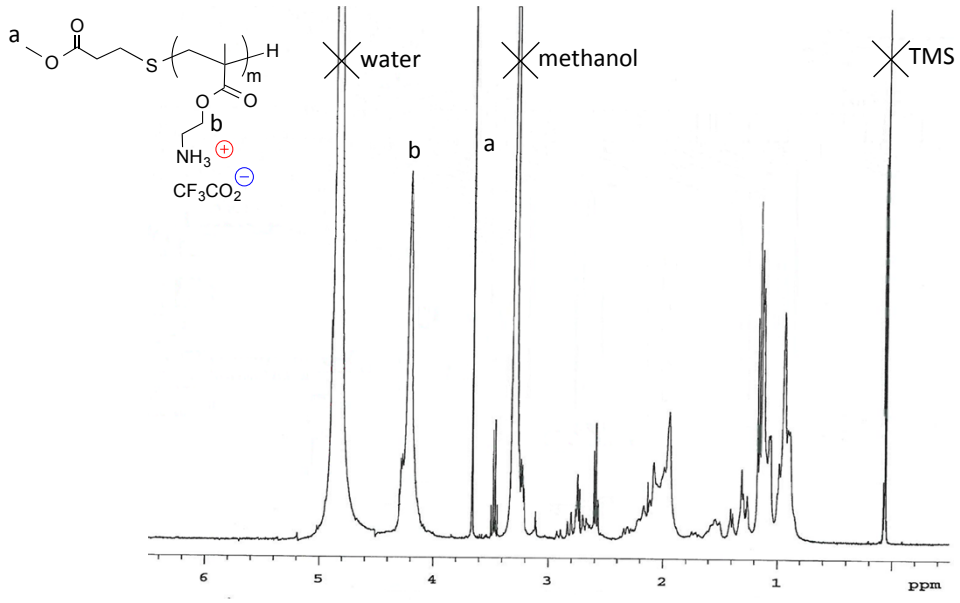

Figure S2. ^{1}H NMR spectrum of PM_0 in methanol-d_4. The degree of polymerization (DP) was determined by comparing integrated peaks of methyl group of chain transfer agent (peak: a) and side chains (peak: b).

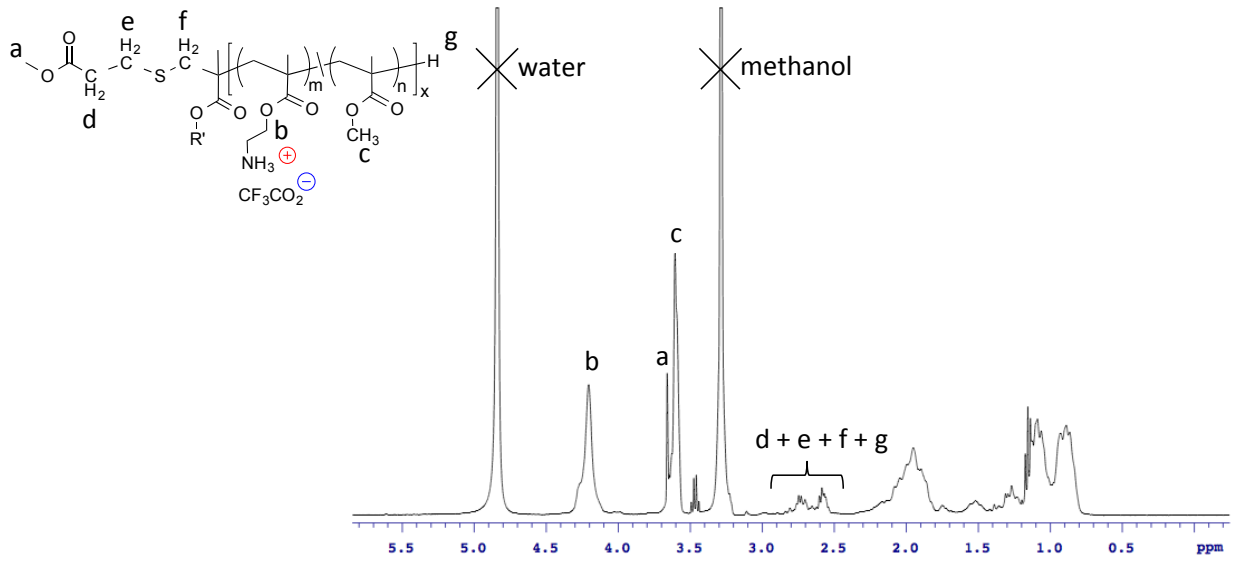

Figure S3. ^{1}H NMR spectrum of PM_{43} in methanol-d_4. The degree of polymerization (DP) and mole percentage of methyl methacrylate group were determined by comparing integrated peaks of methyl group of chain transfer agent (peak: a), the methylene (peaks: d, e, and f), proton in polymer terminus (peak: g) and side chains (peaks: b and c).

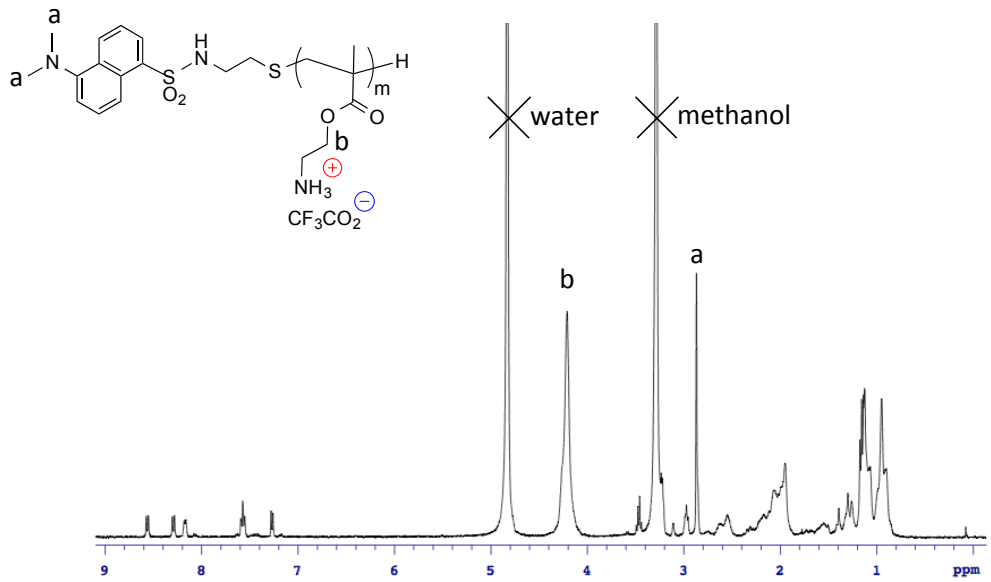

Figure S4. ^{1}H NMR spectrum of D-PM_0 in methanol-d_4. The degree of polymerization (DP) was determined by comparing integrated peaks of methyl group of dancyl group-conjugated chain transfer agent (peak: a) and side chains (peak: b).

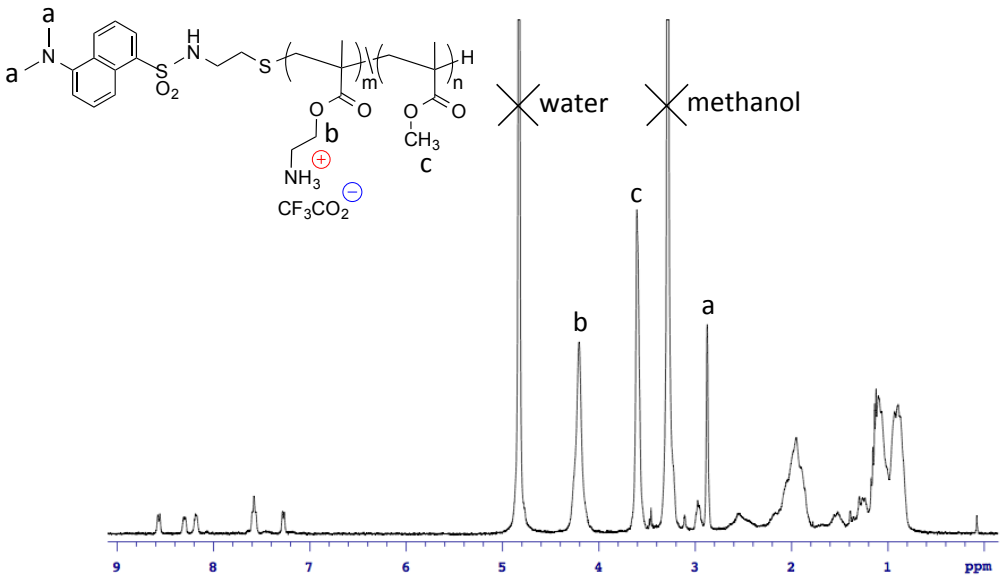

Figure S5. ^{1}H NMR spectrum of D-PM$_{41}$ in methanol-d$_4$. The degree of polymerization (DP) and mole percentage of methyl methacrylate group were determined by comparing integrated peaks of methyl group of dancyl group-conjugated chain transfer agent (peak: a) and side chains (peaks: b and c).

Reverse-phase UPLC. UPLC analysis was performed using a Waters Acquity Peptide Mapping System equipped with a Waters photodiode array detector, a column manager that facilitates 4 column housing, and a sample manager. The instrument is controlled by Empower 3 software. For characterization, copolymers were run on an Acquity BEH C4 column (100 x 2.1 mm, 1.7 □m). The analysis was carried out using a gradient elution beginning with 99:1 (v/v) water/acetonitrile reaching 20:80 water/acetonitrile in 13.40 minutes. 0.14 wt.% trifluoroacetic acid (TFA) was added in water as well as in acetonitrile as a counter ion to make the copolymer surfaces hydrophobic. Flow rate was maintained at 0.200 mL minute^{-1} and the software is equipped with three different injection options. The copolymers were dissolved in nano-pure water at a concentration of ~3 mg mL^{-1}. And a 3 µL of sample was injected using one such option ("partial loop with needle overfill"). The column temperature was maintained at 35 °C. The chromatogram was collected at 285 nm and peaks from nano-pure water was subtracted for background subtraction.

Preparation of CHP and M-β-CD solutions. Cholesterol-bearing pullulan (CHP), substituted with 1.2 cholesterol groups per 100 glucose units of the parent pullulan (MW=100,000) (The degree of substitution of cholesterol groups, DS= 1.2 mole %), was obtained as previously described procedure.[3] CHP was suspended and swollen in distilled water with stirring overnight at room temperature. The suspension was sonicated using a probe-type sonicator (Sonifier 250, Branson Ultrasonics Co.; tip diameter 2 mm) at 40 W for 15 min under cooling with ice, then lyophilized to give a white powder. The resulting powder was re-suspended in PBS buffer (GIBCO®, pH 7.4) at a concentration of 20 mg mL^{-1} and filtered through a 0.22 μm PVDF filter (Millex®-GV, Millipore Co.) to remove impurities and sterilize. Finally, a clear aqueous solution was obtained. As previously reported, the M_w of CHP nanogels was 6.0×10^5, determined by size exclusion chromatography with multi-angle laser light scattering (SEC-MALLS) using 0.142 mL g^{-1} as the value of refractive index increment (d*n*/d*c*).[3, 4] Methyl-β-cyclodextrin was selected as cyclodextrin (CD) for this study instead of β-cyclodextrin, a well-known host-guest agent with cholesterol group, because it showed higher solubility into water and capability to cap the cholesterol group to induce the dissociation of CHP chains compared to β-cyclodextrin.[4] Methyl-β-cyclodextrin (CD) was dissolved in PBS buffer (pH 7.4) at a concentration of 100 mg mL^{-1} with vortex and filtered through a 0.22 μm PVDF filter to remove impurities and sterilize.

Preparation of polymer-CHP complex and addition of M-β-CD. Each antimicrobial polymer was dissolved in DMSO at 20 mg mL^{-1} as a stock solution, and serial dilutions of polymers were prepared from stock solution by dilution with PBS buffer (pH 7.4). The serial polymer dilutions were mixed with the CHP solution and incubated for 30 min at room temperature. The concentration of CHP in this solution was 10 mg mL^{-1}. The polymer–CHP solution was 10-fold diluted with an optical medium (PBS buffer, bacterial or RBCs solution) to give final CHP concentration at 1,000 μg mL^{-1}. Then, 100 mg mL^{-1} of M-β-CD was added to the polymer–CHP solution (1/100 or 1/50 (v/v)) and incubated for 2 h at 37 ºC with orbital shaking (180 rpm). The final concentration of M-β-CD was 1,000 or 2,000 μg mL^{-1}.

MBC assay. The minimum bactericidal concentration (MBC) was defined as the lowest polymer concentration to kill a particular bacterium. The $MBC_{99.9}$ of polymers against *E. coli* were determined as concentration at which 99.9 % of *E. coli* was killed after 1 h incubation in PBS buffer (pH 7.4). An overnight (approximately 18 h) culture of *E. coli* was regrown to exponential

phase (OD_{600} of 0.5–0.7) in Mueller Hinton Broth (MHB, BD and Company©). This bacterial solution was centrifuged at 425 × g for 5 min to give bacterial pellet and the supernatant broth was removed by pipetting. The pellet was suspended in PBS and washed once by another centrifugation. The resulting bacterial suspension was diluted with PBS to OD_{600} of 0.05 to give approximately 2×10^7 cfu mL^{-1} as final concentration. Polymer solutions (10 μL) were prepared by dilution with PBS from stock solution (20 mg mL^{-1} in DMSO) in sterile polystyrene microtubes, then the bacterial suspension (90 μL) was added and incubated at 37 °C with orbital shaking (180 rpm). PBS (10 μL) was used as a control. After 1 h, the solutions were diluted 1000-fold with PBS, streaked onto Mueller Hinton agar plates, and incubated at 37 °C overnight. The colonies were then counted and the number of viable cells as colony-forming unit (cfu) mL^{-1} in the polymer solution was obtained. The MBC was determined as the lowest polymer concentration at which 99.9 % reduction of viable cells was shown compare to control (PBS). Each MBC assay was independently repeated at least three times using different stock solutions in duplicate on different days.

MIC assay. The minimum inhibitory concentration (MIC) of polymers against *E. coli* were determined in a standard microbroth dilution assay according to the Clinical and Laboratory Standards Institute guidelines with suggested modifications by R.E.W. Hancock Laboratory (University of British Columbia, British Columbia, Canada)[5] and Giacometti *et al*.[6] *E. coli* cultured in Mueller Hinton Broth (MHB, BD and Company©) prepared according to manufacturer's instruction. An overnight (approximately 18 h) culture of *E. coli* was regrown to exponential phase (OD_{600} of 0.5–0.7) and diluted with the MHB to give the bacterial suspension with approximately 4×10^5 cfu mL^{-1} as final concentration. After polymer solutions (10 μL) were prepared by dilution with PBS buffer (pH 7.4) from stock solution (20 mg mL^{-1} in DMSO) on a 96-well sterile round-bottom polypropylene plate, the bacterial suspension (90 μL) was added and incubated for 18 h at 37 °C. DMSO/PBS dilution was used as a solvent control. The MIC was defined as the lowest polymer concentration to completely inhibit visible bacterial growth. Bacterial growth was detected at OD_{600} using WPA S800 visible spectrophotometer (Biochrom). Each MIC assay was independently repeated at least three times using different stock solutions in triplicate on different days.

Hemolysis assay. Human red blood cells from healthy donor (RBCs; 1 mL) were suspended in 9

mL of PBS buffer (pH 7.4) and centrifuged at 660 × g for 5 min. The supernatant was removed by pipetting and RBCs were re-suspended in PBS. This procedure was repeated two additional times. The number of RBCs in resulting suspension was counted by counting chamber and diluted in PBS to give 3.0×10^8 cells mL^{-1} as final concentration. After polymer solutions (10 μL) were prepared by dilution with PBS from stock solution (20 mg mL^{-1} in DMSO) on a 96-well sterile round-bottom polypropylene plate, the RBC suspension (90 μL) was added and incubated at 37 °C with orbital shaking (180 rpm). Triton X-100 (0.1 % (v/v) in water) was used as the positive lysis control and PBS was used as negative control. The bee venom toxin melittin was also tested as reference standard. After incubation for 1 h, the plate was centrifuged at 1000 × g for 5 min and supernatant (6 μL) from each well was diluted with PBS buffer (100 μL) in a 96-well sterile flat-bottom polystylene plate. The absorbance of the released hemoglobin at 415 nm was measured using Varioskan Flash microplate reader (Thermo Fisher). The percentage of hemolysis was calculated relative to the positive control Triton X-100 (100 %) and negative control PBS buffer (0 %). The HC_{50} was defined as the polymer concentration causing 50 % hemolysis. The HC_{50} or hemolysis% at highest concentration if the hemolysis% showed below 50 % is reported. Each hemolysis assay was independently repeated at least three times using different stock solutions in triplicate on different days.

Polymer–CHP nanogels binding and releasing experiment. Binding and releasing of random copolymer with dansyl group between CHP nanogels was assayed by monitoring changes in the emission intensity of dansyl. As time 0 min, dansyl-labeled random copolymer D-PM_{41} solution (1.9 mL, 15.8 μg mL^{-1}) put in a cuvette. The cuvette placed in the dark at room temperature with slow stirring. 10 min later, 100 μL of the CHP solution (20 mg mL^{-1}) or PBS buffer (pH 7.4) was added into copolymer solution and incubated for 90 min. The concentration of D-PM_{41} was 15 μg mL^{-1} and that of CHP was 1 mg mL^{-1}. After 90 min incubation, M-β-CD solution (40 μL, 100 mg mL^{-1}) was added, and the fluorescence intensity was monitored for the next 120 min. The final concentration of D-PM_{41}, CHP nanogel and M-β-CD were 14.7 μg mL^{-1}, 980 μg mL^{-1} and 1960 μg mL^{-1}, respectively. The fluorescence intensity was recorded at appropriate time intervals on a fluorescence spectrophotometer (FP-6200, JASCO Co.) using the following conditions: excitation at 330 nm, emission at 510 nm, slit width 5 nm for both of excitation and emission. Each experiment was independently carried out at two times using different stock solutions and obtained same similar profile.

DLS and ζ-potential measurements. The dynamic light scattering **(**DLS) measurements were carried out at 25 °C using a Zetasizer Nano ZS (Malvern Instruments Ltd.) at a wavelength of 633 nm and a 173° detection angle to determine the size of the CHP nanogels and polymer–CHP complexes. The final concentration of homopolymers and random copolymers were 500 μg mL^{-1} and 15 μg mL^{-1}, respectively. The DLS measurements were performed in the absence or presence of M-β-CD at the final concentration of 1,000 or 2,000 μg mL^{-1}. The measured autocorrelation function was analyzed by the cumulant method. The hydrodynamic radius of the particles was calculated using the Stokes-Einstein equation. The ζ-potential measurements were also carried out at 25 °C using a Zetasizer Nano ZS at a 90° detection angle to investigate the ζ-potential of the CHP nanogels and polymer–CHP complexes in PBS buffer (pH 7.4). For the ζ-potential measurement, the concentration of CHP was increased to 4 mg mL^{-1} to obtain enough scattering. The final concentration of random copolymers was 60 μg mL^{-1}, at which the molar ratio of copolymer to CHP was same as the DLS measurement condition. The ζ-potential measurements were performed using a capillary ζ-potential cell in automatic mode. Each measurement was independently repeated at three times using different stock solutions.

Fluorescence measurements. The fluorescence measurements were performed to estimate association and dissociation between dansyl-labeled random copolymers and CHP nanogels. The final concentration of copolymers and CHP nanogels were 15 μg mL^{-1} and 1,000 μg mL^{-1}, respectively.

To polymer or polymer–CHP complex solution, the M-β-CD (100 mg mL^{-1}) was added to give the final concentration at 1,000 or 2,000 μg mL^{-1}, and incubated for 2 h at 37 ºC with orbital shaking (180 rpm). The fluorescence intensities were measured using excitation at 330 nm and emission at 510 nm. Each measurement was independently repeated at three times using different stock solutions.

Polymer–CHP dissociation constant measurement. Polymer with dansyl group binding to CHP nanogels was assayed by monitoring changes in the emission intensity of dansyl. An aliquot of the CHP solution (2.5 μL or 5 μL, 20 mg mL^{-1}) was added into a cuvette containing polymer solution (3 mL, 1 μM), and the fluorescence intensity was recorded after 5 min of mixing. The fluorescence intensity was recorded on a fluorescence spectrophotometer (FP-6200,

JASCO Co.). The fluorescence data were corrected for dilution as well as the inner-filter effect. The measured fluorescence intensities were plotted versus the total concentration of CHP nanogel, and the dissociation constant (K_d) was analyzed by curve-fitting to the following equation (S1) for single-site binding isotherm using KaleidaGraph software package:

$$F = F_0 + \Delta F \frac{K_d + [P]_T + n[C]_T - \sqrt{(K_d + [P]_T + n[C]_T)^2 - 4n[P]_T[C]_T}}{2[P]_T} - (S1)$$

where $[P]_T$ and $[C]_T$ are total polymer and CHP nanogel concentrations, respectively, F_0 is the fluorescence intensity before the addition of CHP nanogel ($[C]$=0), ΔF is the change in fluorescence intensity from F_0 to the fluorescence when the CHP nanogel concentration approached infinity, and n is the number of binding site per one CHP nanogel. n was tested from 1 to 20 and good data fitting was obtained using n=13–18. Each assay was independently repeated at three times using different stock solutions.

Bactericidal kinetics. Time-kill studies were performed to examine the bactericidal activity of copolymers was examined according to the capture and release of copolymers from the CHP nanogels. A regrown *E. coli* was suspended in PBS buffer (pH 7.4) at OD_{600} of 0.05 to give approximately 2×10^7 cfu mL^{-1} as final concentration as well as MBC assay. As time 0 min, PBS (80 μL) was added to the resulting bacterial suspension (720 μL) in a sterile polystyrene microtube, and incubated at 37 °C with orbital shaking (180 rpm). After 10 min, non-labeled random copolymers and copolymer-CHP complexes (80 μL) were added to the bacterial suspension (720 μL) in another microtubes and incubated. The final concentration of copolymers and CHP nanogels were 15 μg mL^{-1} and 1,000 μg mL^{-1}, respectively. 60 min later, 500 μL of copolymer−CHP complex solution was moved to a new microtube, and M-β-CDs (10 μL, 100 mg mL^{-1}) was added and incubated for 120 min. Aliquots from each solution were drawn at appropriate time intervals and immediately diluted with PBS at least 1000-fold to remove the effects of the copolymer. The solutions were then streaked onto Mueller Hinton agar plates, and incubated at 37 °C overnight. The colonies were counted and the number of viable cells as colony-forming unit (cfu) mL^{-1} was obtained. The percentage of bacteria viability was calculated relative to the PBS buffer at 0 min (100 %). Each experiment was independently repeated at three times in duplicate on different days.

ESI 2. Characterization of Boc-protected methacrylate random copolymers.

Table S1. Characterization of Boc-protected copolymers.

	CTA[a]	MP_{methyl} (mol. %)[b]	DP[c]	M_n, NMR[d]	M_n, GPC[e]	M_w, GPC[e]	$Đ$[f]
PM_0	MMP	0	12	3,000	2,400	3,000	1.24
PM_{43}	MMP	45	15	2,600	2,100	2,500	1.18
D-PM_0	Dansyl	0	10	2,700	1,900	2,500	1.31
D-PM_{41}	Dansyl	43	10	2,000	1,600	2,000	1.27

[a] CTA: chain transfer agent. Methyl 3-mercaptopropionate (MMP) or dansyl group-conjugated chain transfer agent (Dansyl) was used.

[b] Mole percent of methyl group in a polymer chain determimed by ^{1}H NMR.

[c] The number average degree of polymerization (DP) determimed by ^{1}H NMR.

[d] the number average molecular weight (M_n) calculated based on the molecular weight of monomers, mole percent of methyl groups (MP_{methyl}) and DP.

[e] The number average molecular weight (M_n), The weight average molecular weight (M_w) determimed by GPC. The molecular weight calibration was based on poly(methyl methacrylate) standards.

[f] Polydispersity index ($Đ$) was calculated as M_w/M_n using M_w and M_n values determined by GPC.

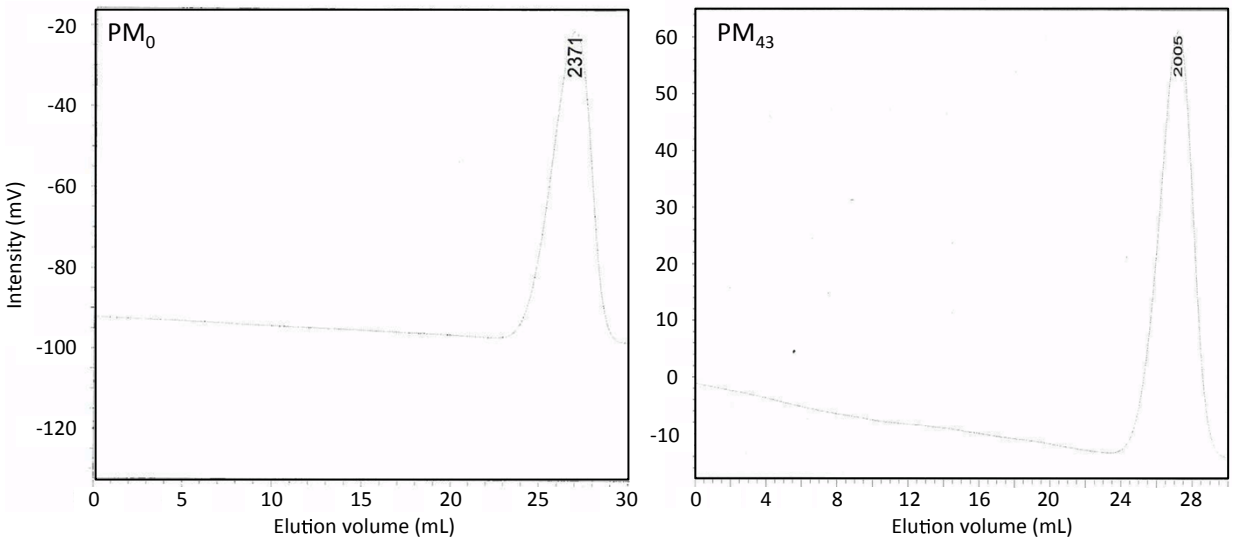

Figure S6. Gel permeation chromatographs of PM_0 (left) and PM_{43} (right) in THF.

ESI 3. Reverse-phase UPLC analysis of de-protected copolymers.

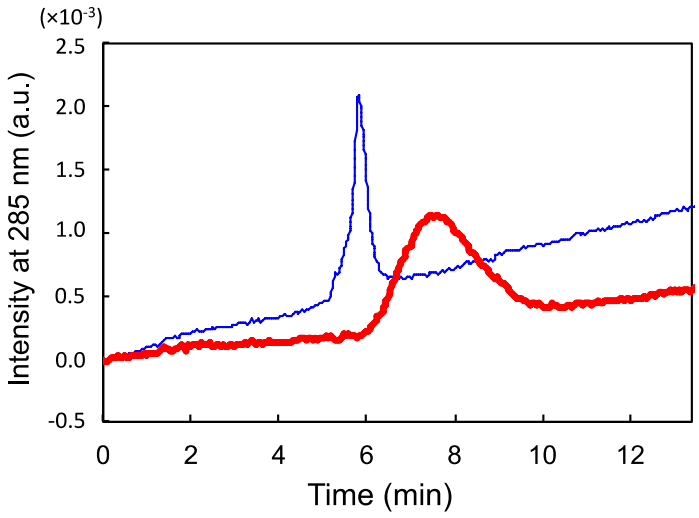

Figure S7. Reverse-phase UPLC chromatographs of PM_0 (blue thin line) and PM_{43} (red thick line).

ESI 4. Antimicrobial activity of copolymer–CHP complex.

Table S2. Antimicrobial activity of copolymer–CHPs in the presence and absence of CD.

	MIC (μg mL^{-1})[a]	MBC$_{99.9}$ (μg mL^{-1})[b]
PM$_{43}$	83 ± 4	14 ± 2
PM$_{43}$ + CHP	567 ± 47	78 ± 12
CHP	>2,000	>2,000
CD	>4,000	>4,000

[a] Minimum inhibitory concentration against *E. coli* in Mueller-Hinton broth.

[b] Minimum bactericidal concentration producing 99.9 % killing within 1 h against *E. coli* in PBS (pH 7.4). The final cocentration of CHP in the test solutions was [CHP]= 1,000 μg mL^{-1} except the assays for CHP.

ESI 5. DLS and ζ-potential measurements of copolymer–CHP complex.

Table S3. Size and ζ-potential of copolymer–CHP complex in the presence and absence of CDs.

	[CD] (μg mL^{-1})[a]	D_H (nm)[b]	PDI[b]	ζ-potential (mV)
PM$_0$	0	N.D.[c]	N.D.[c]	N.D.[c]
PM$_{43}$	0	N.D.[c]	N.D.[c]	N.D.[c]
PM$_0$ + CHP	0	50.6 ± 1.6	0.255	n.d.
PM$_{43}$ + CHP	0	49.9 ± 1.4	0.263	-0.7 ± 0.4
PM$_0$ + CHP + CD	1,000	45.9 ± 1.0	0.403	n.d.[d]
	2,000	45.1 ± 0.8	0.483	n.d.[d]
PM$_{43}$ + CHP + CD	1,000	44.4 ± 0.5	0.406	n.d.[d]
	2,000	43.3 ± 0.2	0.483	n.d.[d]
CHP	0	48.7 ± 1.7	0.237	-1.1 ± 0.2
CD	2,000	N.D.[c]	N.D.[c]	N.D.[c]

[a] [CD]: the final concentration of methyl-β-CD (CD) in the test solutions.

[b] Hydrodynamic diameter (D_H) and polydispersity index (PDI) measured by DLS. The final cocentration of materials in the test solutions were; [PM$_0$]= 500 μg mL^{-1}, [PM$_{43}$]= 15 μg mL^{-1},

and [CHP]= 1,000 μg mL^{-1}.

[c] Not detectable even at highest concentration (1,000 or 2,000 μg mL^{-1}) because their sizes were too small to be detected (standard detection limit= >10−15 nm in diameter).

[d] Not determined.

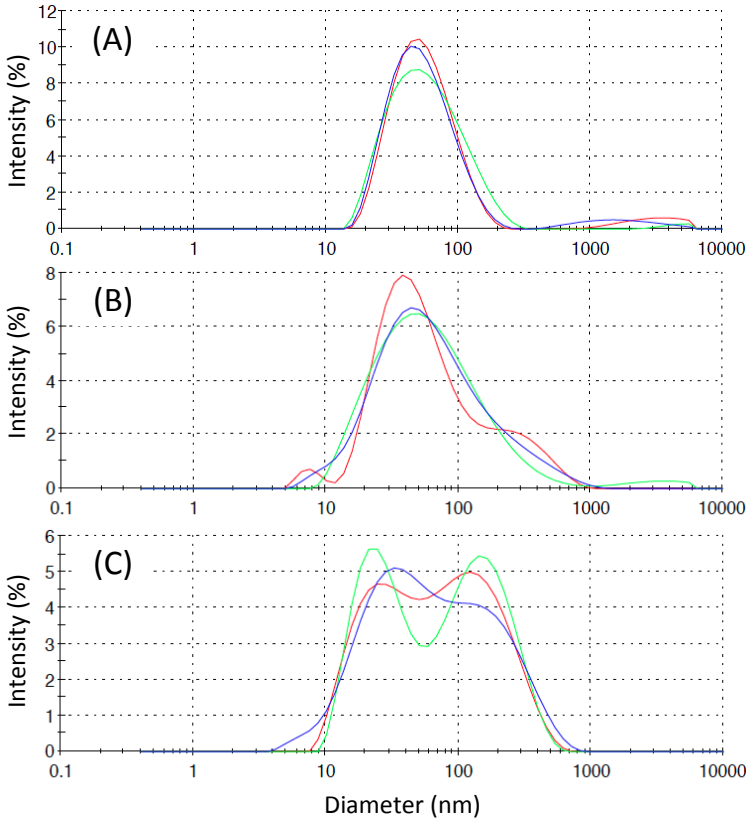

Figure S8. The scattering intensity distribution of PM_{43}+CHP complex and PM_{43}+CHP+CD. (A) PM_{43}+CHP complex. [PM_{43}]= 15 μg mL^{-1}, [CHP]= 1,000 μg mL^{-1}. (B) PM_{43}+CHP+CD. [CD]= 1,000 μg mL^{-1}. (C) PM_{43}+CHP+CD. [CD]= 2,000 μg mL^{-1}.

ESI 6. Releasing of copolymers from CHPs induced by CD.

The releasing of copolymer was evaluated by fluorescence measurements using dansyl-labeled copolymers shown in Figure S6 as well as Figure 3. By binding CHP, the fluorescence intensity from dansyl-group was abundantly increased. CD addition induced the decreasing of fluorescence intensity, and amount of released copolymer was dependent on CD concentration. At [CD]= 1,000 μg mL^{-1}, the value of fluorescence intensity of D-PM_{41}–CHP–CD

was bigger than that of D-PM_{41}–CD. However, in the case of 2,000 μg mL^{-1}, the value of fluorescence intensity of D-PM_{41}–CHP–CD reached to same level as D-PM_{41}–CD.

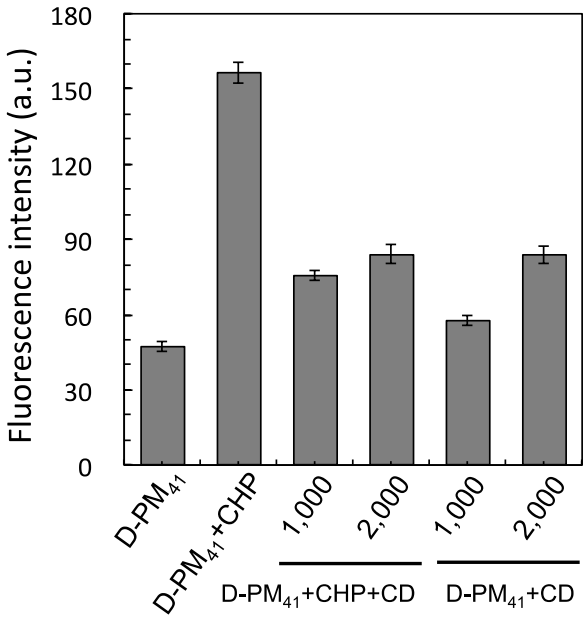

Figure S9. Releasing of dansyl-labeled copolymers from CHPs induced by CD. The D-PM_{41} was incubated with CHPs for 30 min in PBS (pH 7.4). Then CDs were added and incubated for 2 h at 37 ºC. Final concentrations; [D-PM_{41}]= 15 μg mL^{-1}, [CHP]= 1,000 μg mL^{-1}, and [CD]= 1,000 or 2,000 μg mL^{-1}.

ESI 7. Copolymer–CHP dissociation constant measurement.

In order to quantify binding affinity between copolymers and CHP nanogels, the dissociation constant (K_d) was determined using fluorescent proves (Figure S10). We previously demonstrated that the dissociation constant of copolymers to lipid membranes could be measured using the dansyl-group conjugating copolymers[1, 2] as well as Hercules *et al*.[7] We expected this methodology could assess to determine the dissociation constant of copolymers to CHPs. The emission intensity from the dansyl-group increased with titration of CHPs in the case of incubation with amphiphilic copolymers D-PM_{41}. The obtained binding isotherms fitted to the following equation (S2);

$$F = F_0 + \Delta F \frac{K_d + [P]_T + n[C]_T - \sqrt{(K_d + [P]_T + n[C]_T)^2 - 4n[P]_T[C]_T}}{2[P]_T} \quad -(S2)$$

where $[P]_T$ and $[C]_T$ were total polymer and CHP nanogel concentrations, respectively, F_0 was the fluorescence intensity before the addition of CHPs ($[C]$=0), ΔF was the change in fluorescence intensity from F_0 to the fluorescence when the CHP concentration approached infinity. We hypothesized that the CHP nanogel was capable to incorporate multiple polymers, thus put n which was the number of binding site per one CHP nanogel. A good data fitting was obtained using n=13–18 which indicated that 13−18 of copolymer chains bound to one nanogel. The dissociation constant (K_d) of D-PM$_{41}$ to one binding site of CHPs was K_d= 1.1 ± 0.4 μM. However, when the cationic homopolymer D-PM$_0$ was incubated, no significant increase of emission intensity was detected, indicating the homopolymer didn't bind to CHPs.

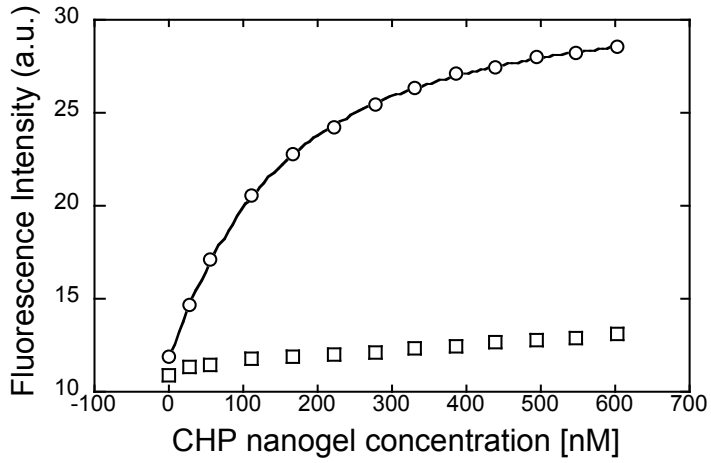

Figure S10. Representative binding isotherms of dansyl-labeled polymers to CHPs: homopolymer D-PM0 (□) and copolymer D-PM41 (●). The assay was independently repeated at three times using different stock solutions to give the average dissociation constant ± standard deviation (S. D.). [dansyl-labeled polymer] = 1 mM.

ESI 8. Bacteria viability in the presence of CHPs and cyclodextrins.

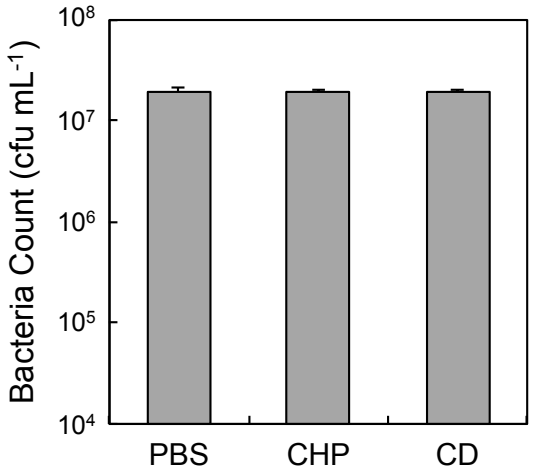

Figure S11. Bacteria viability in the presence of CHP and methy-β-CD (CD) after incubation with *E. coli* for 1 h in PBS (pH 7.4). Detection limit of bacterial concentration was 1×10^4 cfu mL^{-1}. [CHP]= 2,000 mg mL^{-1}, [CD]= 4,000 mg mL^{-1}.

ESI 9. Calculation of concentration of bound copolymers upon CHPs.

In general, the binding reaction of copolymers PM_{43} to CHP nanogels was represented by as following;

$$polymer + binding\ site \rightleftarrows complex$$

Hence the CHP nanogel was capable to incorporate multiple polymers, CHP contained multiple binding site contributing to bind to one polymer. The dissociation constant K_d was defined according to the following equation (S3);

$$K_d = \frac{[P] \cdot [B]}{[complex]} \quad -(S3)$$

where $[P]$, $[B]$, [complex] were concentrations of polymer, binding site and PM_{43}–binding site complex, respectively. Each was expressed by equations (S4)−(S6);

$$[P] = [P]_T - [complex] \quad -(S4)$$

$$[B] = [B]_T - [complex] \quad -(S5)$$

$$[B]_T = n[C]_T \quad -(S6)$$

in here $[P]_T$, $[B]_T$, and $[C]_T$ were total concentration of polymer, binding site, and CHP nanogels

in the solutions respectively. n was the number of binding site per one CHP nanogel. Finally the concentration of PM_{43}–binding site complex, [complex], was given by following equation (S7);

$$[comlex] = \frac{K_d + [P]_T + n[C]_T - \sqrt{(K_d + [P]_T + n[C]_T)^2 - 4n[P]_T[C]_T}}{2} \quad -(S7)$$

Under the condition of CHPs captured PM_{43} at [PM_{43}]= 15 μg mL^{-1}, the concentration of captured polymer was calculated using K_d= 1.1 μM and n=13−18 estimated by the fluorescent measurement, and given as [complex]= 4.55−4.64 μM (14.1−14.4 μg mL^{-1}). The concentration of total copolymer $[P]_T$ was 4.84 μM, that meant concentration of free copolymers was 0.2−0.29 μM (0.6−0.9 μg mL^{-1}).

ESI 10. Bacteria viability in the presence of copolymer–CHP complex.

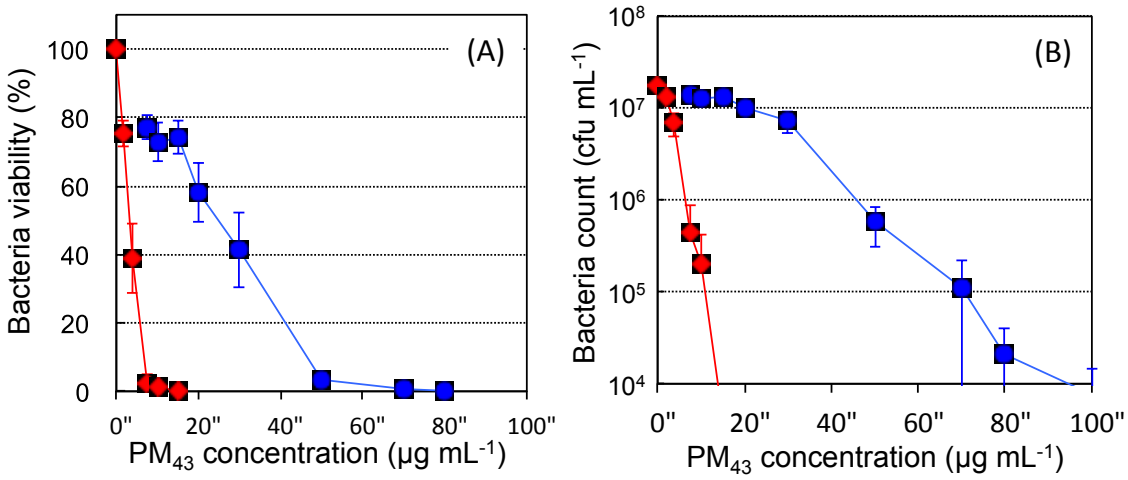

Figure S12. Bacteria viability (A) or Bacteria count (B) in the presence of copolymer PM_{43} (♦) or PM_{43}–CHP complex (●) as a function of PM_{43} concentration after incubation with *E. coli* for 1 h in PBS (pH 7.4). Detection limit of bacterial concentration was 1 × 10^4 cfu mL^{-1}. The final cocentration of CHP was [CHP]= 1,000 μg mL^{-1}.

ESI 11. Recovery of antimicrobial activity of copolymers by addition of CD.

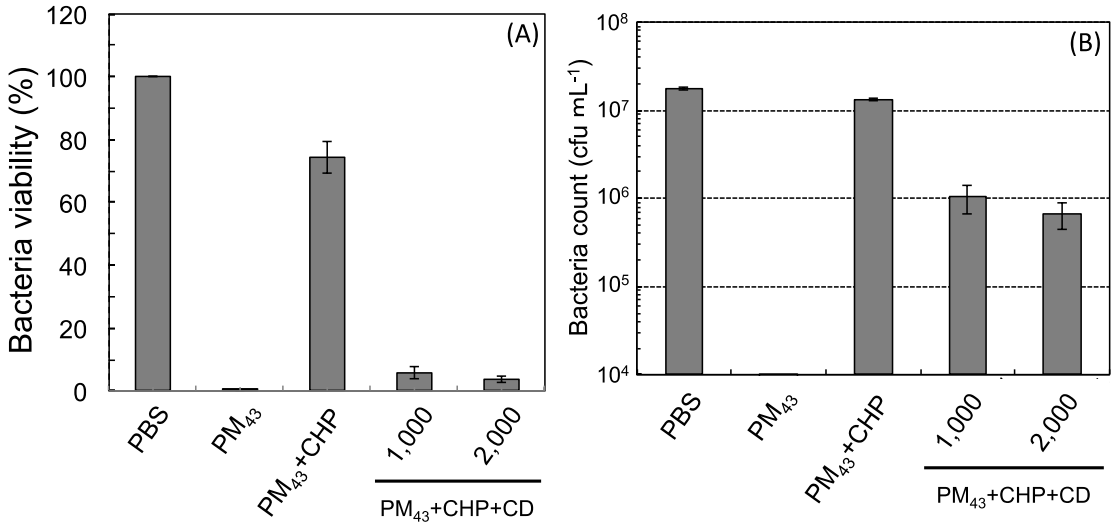

Figure S13. Recovery of antimicrobial activity of copolymers against *E. coli* by addition of methyl-β-cyclodextrins (CDs). (A) bacteria viability (B) bacteria count. *E. coli* was incubated with PM_{43} or PM_{43}–CHP complex for 1 h at 37 ºC in PBS (pH 7.4). Then CDs were added and incubated for 2 h at 37 ºC. Final concentrations; [PM_{43}]= 15 μg mL^{-1}, [CHP]= 1,000 μg mL^{-1}, and [CD]= 1,000 or 2,000 μg mL^{-1}.

ESI 12. Suppression and recovery of antimicrobial activity of copolymers (Bacteria count).

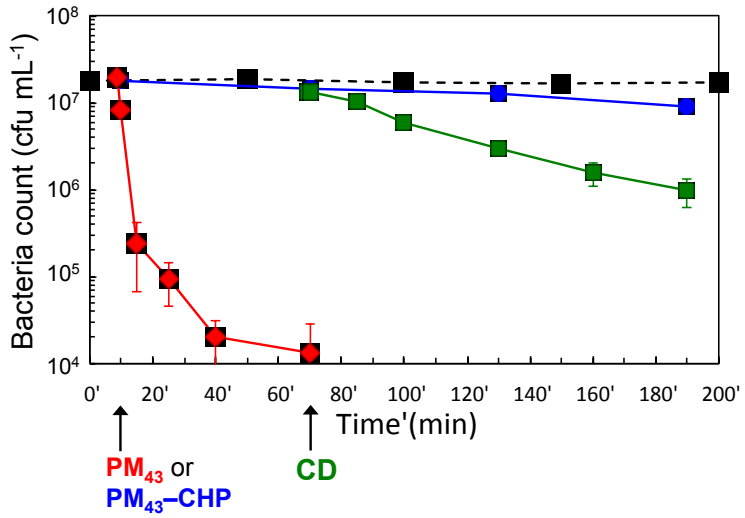

Figure S14. Suppression and recovery of antimicrobial activity of copolymers against *E. coli* (Bacteria count). *E. coli* was incubated at 37 ºC in PBS (pH 7.4). At the time of 10 min, PM_{43} (♦) or PM_{43}–CHP complex (●) were added. At 70 min, CD was added to PM_{43}–CHP complex (■), and the number of viable cells was determined with time; PBS (▲), PM_{43} (♦), PM_{43}–CHP complex (●), and PM_{43}–CHP–CD (■). The final concentrations; [PM_{43}]= 15 μg mL^{-1}, [CHP]= 1,000 μg mL^{-1}, and [CD]= 2,000 μg mL^{-1}. Detection limit of bacterial concentration was 1×10^4 cfu mL^{-1}.

ESI 13. Interference effect of CHP–CD complex against antimicrobial activity of copolymers.

To examine an interference effect of CHP–CD complex against the bactericidal activity of PM_{43} copolymers, the time-kill studies were performed. CHP nanogel was first incubated with CD for 120 min to dissociate CHP chains forming CHP–CD complex. At the time of 10 min, the PM_{43} copolymers was mixed with CHP–CD complex, then immediately the solution was added to the bacterial suspension and incubated for 60 min at 37 °C with orbital shaking (180 rpm). The final concentration of copolymers, CHP nanogels, and CD were 15 μg mL^{-1}, 1,000 μg mL^{-1}, and 2,000 μg mL^{-1}, respectively. Aliquots from each solution were drawn at appropriate time intervals, streaked onto Mueller Hinton agar plates, and incubated at 37 °C overnight. The colonies were counted and the number of viable cells as colony-forming unit (cfu) mL^{-1} was obtained. The percentage of bacteria viability was calculated relative to the PBS buffer at 0 min (100 %). Each experiment was independently repeated at three times in duplicate on different days.

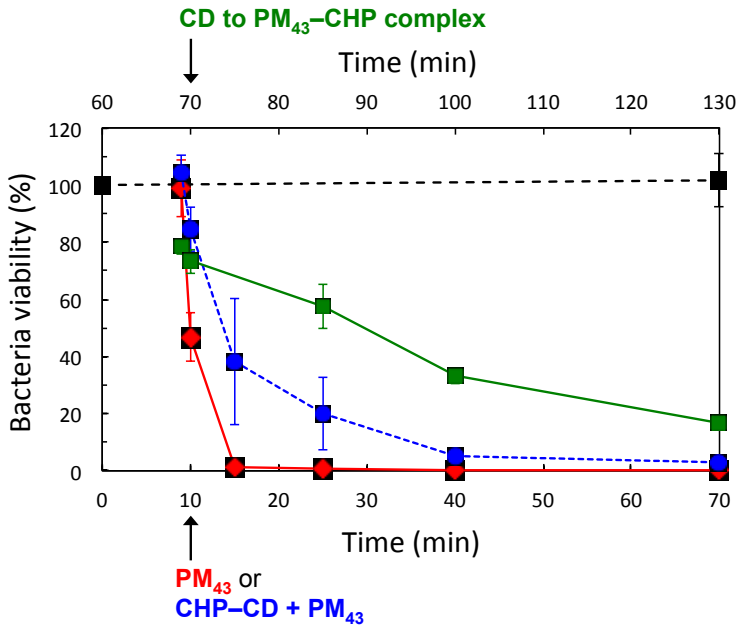

Figure S15. Interference effect of CHP–CD complex against bactericidal activity of copolymers. CHP nanogel was first incubated with CD for 120 min to dissociate CHP chains forming CHP–CD complex. *E. coli* was incubated at 37 ºC in PBS (pH 7.4). At the time of 10 min, PM_{43} was mixed with CHP–CD complex (CHP–CD + PM_{43}) (●), then immediately the solution was added to the bacterial suspension, and the number of viable cells was determined with time. Addition of PM_{43} (♦) at 10 min, which is only copolymer, is reprinted from Figure 4. PM_{43}–CHP complex added CD solution (■) at 70 min is also reprinted from Figure 4 using upper x axis; PBS (▲), PM_{43} (♦), PM_{43}–CHP–CD (■), and CHP–CD + PM_{43} (●). The final concentrations; [PM_{43}]= 15 μg mL^{-1}, [CHP]= 1,000 μg mL^{-1}, and [CD]= 2,000 μg mL^{-1}.

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