Supporting Information

Quaternization-induced Micellization of Cationic Glycopolymers

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Experimental:

Materials

4-vinylpyridine (4VP, 99%), methacrylate (MA), 2-(Diethylamino)ethyl methacrylate (DEAEMA), 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU), copper wire, ethyl 2-bromoisobutyrate (EBiB), and copper bromide ($CuBr_2$) were purchased from Shanghai Aladdin Bio-Chem Technology Co., Ltd. ε-caprolactone (CL, Aladdin, 99.9%) and toluene were dried over calcium hydride at room temperature for 48 h and then was distilled under pressure before use. Tris(2-dimethylaminoethyl)amin (Me_6TREN), 2-hydroxyethyl 2-chloropropanoate (HECP) were prepared following previously described route elsewhere.[1] 2-Bromoethanol was purchased from Shanghai Aladdin Bio-Chem Technology Co., Ltd. (*3R,4S,5S,6R*)-2-(3-bromopropoxy)-6-(hydroxymethyl)tetrahydro-*2H*-pyran-3,4,5-triol (A_3-GluBr) was prepared following previously described route elsewhere.[2] Gram-negative bacterium *E. coli* (ATCC25922) and Gram-positive bacterium *S. aureus* (ATCC29223) were purchased from Nanjing Bianzhen Biological Technology *Co. Ltd*. The inhibitor of 4VP was removed by filtration through a basic alumina column before use. Other chemicals were used as they were purchased. Tetrahydrofuran (THF, Aladdin, 99.5%, water ≤ 50 ppm) was dried over molecular sieves (4 Å) before use.

Synthesis of bromoalkyl glycosides

D-Glucopyranose penta-O-acetate (0.513g, 1.31 mmol 1.0 equiv), 3-bromoethanol (1.06 g, 1.41 mmol, 1.0 equiv), molecular sieves 4Å (1.00 g), and CH_2Cl_2 (3.0 mL) was added in a vial. Then $BF_3 \cdot OEt_2$ (1.0 mL, 7.96 mmol, 5.9 equiv) was then slowly added to the reaction solution. The reaction was stirred for 12 hours at room temperature. The crude residue was purified by column chromatography to give product (*2R,3R,4S,5R*)-2-(acetoxymethyl)-6-(3-bromopropoxy)tetrahydro-*2H*-pyran-3,4,5-triyl triacetate (0.272 g, 0.597 mmol) in 45% yield as white solid. Intermediate compound (0.272 g, 0.597 mmol), dry MeOH (2.0 mL), and NaOMe (1 mL, 5 M) was stirred for 5 hours at room temperature. The crude residue was purified by column chromatography to give final product (167.2 g, 0.555 mmol) in 93% yield;

Synthesis of the macroinitiator polycaprolactone (PCL-Cl) *via* ring opening polymerization

A round-bottom flask previously dried in oven at 90 ^oC overnight was equipped with a magnetic stir bar and a rubber stopper, and then inflated with nitrogen before use. 20 mL (0.18 mol) of ε-CL and 15 mL of ultra-dried THF were degassing with nitrogen and then carefully added to the flask *via* a syringe. 14.4 μL of DBU (0.045 mmol) was added before adding 274.6 mg (1.8 mmol) of 2-hydroxyethyl 2-chloropropanoate dried over molecular sieves (4 Å). The reaction was last for 12 h at the temperature, and stopped by putting it into liquid nitrogen. The polymer was purified with precipitation in in a

huge quantity of cold heptane for three times, and dried under reduced pressure to obtained white powder. Yield = 99%.

Synthesis of the PCL-*b*-P4VP *via* Cu(0)-RDRP

315.4 mg (3 mmol) of 4VP, 9.9 mg of $CuCl_2$ (0.1 mmol), 399.2 mg of PCL-Cl (0.1 mmol) and 3 mL of DMSO were added to a schlenk tube. The mixture was bubbled with high purity N_2 for 15 min. 42.5 μl of pre-degassed Me_6TREN (40.9 mg, 0.11 mmol) were added to above mixture *via* syringe sequentially. After that, pre-activated copper wire (3 cm, 13 mg) treated with hydrochloric acid was carefully added under nitrogen protection. The mixture was bubbled with high purity N_2 for another 15 min. The reaction was allowed to polymerize at 25 °C for 12h. The polymer was purified with precipitation in in a huge quantity of cold diethyl ether for three times, and dried under reduced pressure to obtained white powder. Yield = 88%.

Synthesis of the PMA-*b*-PDEAMEA *via* Cu(0)-RDRP

3.63 mL of MA (40 mmol), 22.3 mg of $CuBr_2$ (0.10 mmol), 0.14 ml of EBiB (1 mmol) and 5.5 mL of DMSO were added to a Schlenk tube. The mixture was bubbled with high purity N_2 for 15 min. 48 μl of Pre-degassed Me_6TREN (0.18 mmol) were added to above mixture *via* syringe sequentially. After that, pre-activated copper wire (3 cm) treated with hydrochloric acid was carefully added under nitrogen protection. The mixture was bubbled with high purity N_2 for another 15 min. This reaction was allowed to polymerize at 25 °C for 12h. 4.03 mL of pre-degassed DEAEMA in 3 mL DMSO

added to above polymerized mixture for chain extension. After reaction for overnight, the obtained solution was directly transported into one dialysis tubing (MWCO 1000Da) for dialysis against pure water for two days. Yield = 92%.

Synthesis of glyco-micelle *via N*-quaternization-induced self-assembly in aqueous solution

In a typical reaction, PCL_{35}-*b*-$P4VP_{13}$ (20 mg, ~0.05 mmol 4VP unit), A_2-GluBr (41.8 mg, 0.15 mmol), and 10 mL water were added to a 30 mL vial equipped with a magnetic stir rotor. The resulting mixture bubbled for 15 min with N_2 and stirred at 80 °C for defined time. Excess A_2-GluBr was removed by dialysis against water for one day then stored for later use. The concentration of glycol-micelles calculated by drying-weighting method was 1.35 mg/mL. The quaternization reaction of other polymer precursors is in the same way.

Antibacterial test and antibacterial kinetic assay

The minimum inhibitory concentrations (MICs) were determined using a minor modified version of the standard broth dilution method.[3-4] Stock solutions of several cationic glycol-micelle were produced in MHB medium, and initial concentration was set to 1024 μg mL^{-1} or 1500 μg mL^{-1}, respectively. The solutions were serially diluted in MHB medium two times, and 100 μL of each dilution was injected in each well of 96-well microplates (Corning, ThermoScientific), followed adding with 100 μL of bacterial suspension. The value of OD_{600} was measured using a microplate reader spectrophotometer (SpectraMax, M3, Molecular Devices, CA, USA). There was a positive control with no polymer and a negative control with no microorganisms. The MIC was defined as the lowest concentration of a glycol-micelle that inhibited bacteria growth by more than 90%. All tests were conducted in three separate tests, each with a duplicate.

Antibacterial kinetic assay was the same as the above-mentioned cultivation method. Stock of several cationic glycol-micelle at a concentration of MIC were produced in MHB medium. The solutions were serially diluted in MHB medium three times, and 100 μL of each dilution was injected in each well of 96-well microplates (Corning, ThermoScientific), followed adding with 100 μL of bacterial suspension. The value of OD_{600} was recorded by a microplate reader spectrophotometer (SpectraMax, M3, Molecular Devices, CA, USA) at a time of 0, 20, 60, 120, 180, 240, 405, 600 min.

Lectin-binding assay

The turbidimetric assay was adopted to measure the binding ability with ConA *via* dynamic light scattering (DLS).[5] ConA (1 mg mL^{-1}) was fully dissolved in HBS buffer (HEPES 10 mM, NaCl 150 mM, and $CaCl_2$ 1 mM, adjusted to pH 7.4 and filtered with 0.2 μm nylon filters). ConA's exact concentration was determined by using the UV absorbance at 280 nm [A = 1.37 × (mg mL^{-1} ConA)]. The various glycol-micelle (500 μL, 1 mg / mL) was mixed for 12 hours. And then the particle size of aggregation was measured by DLS analysis for comparison with the previous glycol-micelle size. Photograph at different times were also recorded to determine deposition velocity of the aggregation.

Hemolysis test

Stock glycol-micelle at a 1 mg / mL (for PCL-*b*-P4VP) or 1 mg / mL (for PMA-*b*-PDEAEMA) concentration, serially diluted by 2-fold, and 100 μL of each dilution was placed in a 96-well plate.[3] Fresh sterile defibrinated sheep's blood was dispersed in PBS at a concentration of 8% (v/v), and 100 μL of the blood suspension was added to each compound solution. The plate was incubated to allow the glycol-micelle interact with the blood cells at 37 °C for 1 hour. Each mixed sample was centrifuged for 5 minutes at 6500 rpm, and the optical absorbance of supernatant was measured using a UV microplate reader at 570 nm wavelength (SpectraMax, M3, Molecular Devices, CA, USA). The same concentration of blood suspension was added to 0.1 percent (v/v PBS) Triton X-100 as a positive control and blank PBS as a negative control. The hemolysis rate was calculated as $[(A - A_0)/(A_{total} - A_0)] \times 100\%$, where A, A_0, and A_{total} represent the OD_{570} of the supernatant from the centrifugal sample, negative and positive control, respectively.

Characterization

^{1}H NMR spectra were recorded with a Bruker AV 500M spectrometer using deuterated solvents obtained from Aladdin. Monomer conversion for 4VP was calculated by comparing the integral of vinyl protons from the polymer backbones. Fourier transform infrared (FTIR) spectra were recorded on a Nicolet iS5 FTIR spectrometer using an iD7 diamond attenuated total reflectance optical base. The binding activity of sugar-containing polymers with ConA was tested by measuring the absorbance at 420 nm using a SHIMADZU UV-2600 UV/Vis spectrophotometer. The number-average molecular weight (M_n) and molecular weight distribution (M_w / M_n) were determined by Waters 1515 size exclusion chromatography (SEC) in *N, N*-dimethylformamide (DMF) at 40 °C with a flow rate of 1.00 mL min^{-1}, which was equipped with 2414 refractive index (RI) and 2489 UV detectors, a 20 μm guard column (4.6 mm × 30 mm, 100-10K) followed by three Styragel columns (HR_1, HR_3, and HR_4) and autosampler. Narrow linear polystyrene standards in the range of 540 to 7.4 × 10^5 g mol^{-1} were used to calibrate the system. All samples were passed through 0.22 μm nylon filter before analysis. A Malvern Z90 Zetasizer was used to characterize the hydrodynamic size of the self-assemblies. The scattering light at 90° angle was detected and used to analyze the size and distribution. The glycol-micelle's morphology was characterized by transmission electron microscope (TEM, FEI Talos F200x, USA). The polymer morphology was characterized by Transmission electron microscope (TEM, FEI Talos F200x, USA). TEM samples were prepared by drop-casting aliquots (ca. 10 μL) of the solutions onto carbon-coated copper grids which were placed on a piece of

filter paper to absorb excess solvent and dry in the air. Then 10 μL of phosphotungstic acid solution (1%) was drop-casting on the copper grids, and rinse gently three times with pure water after a few minutes.

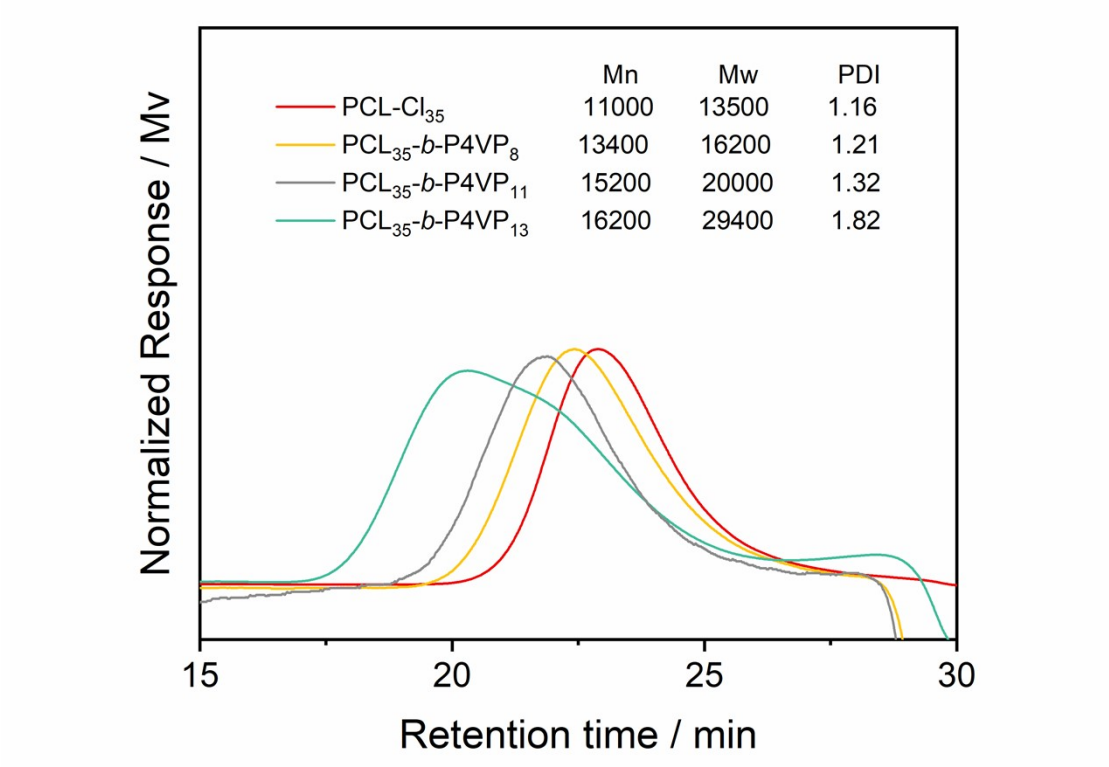


Figure S1. The GPC of macro ATRP initiator (PCL-CL), and block polymer (PCL-*b*-P4VP).

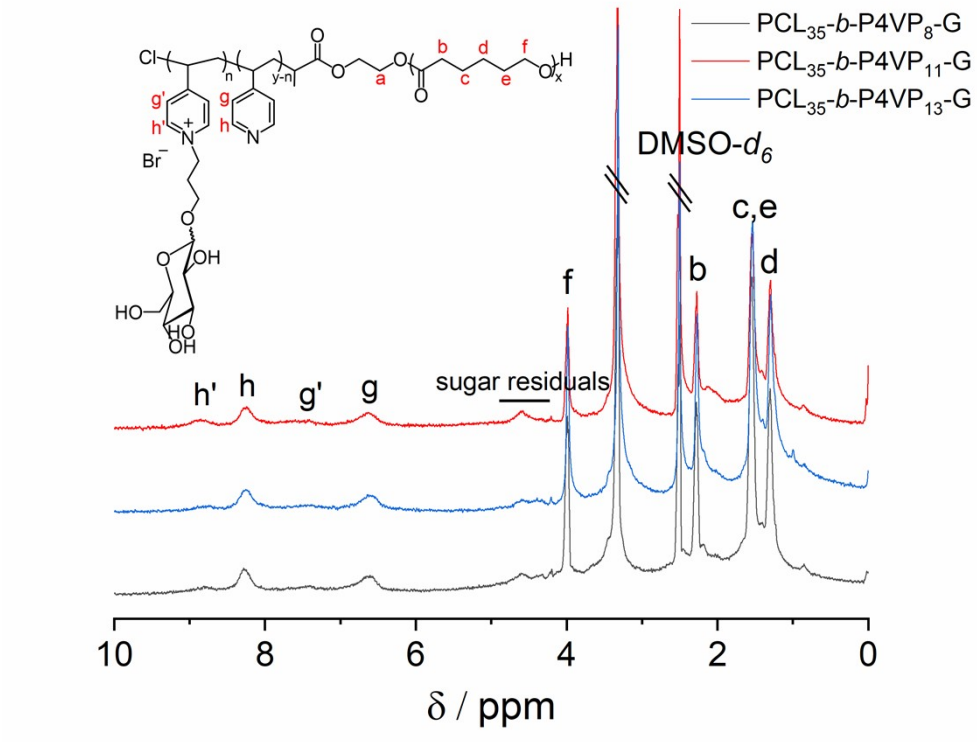


Figure S2. The ^{1}H NMR of various PCL-*b*-P4VP-G using DMSO-d_6 as deuterated solvent .

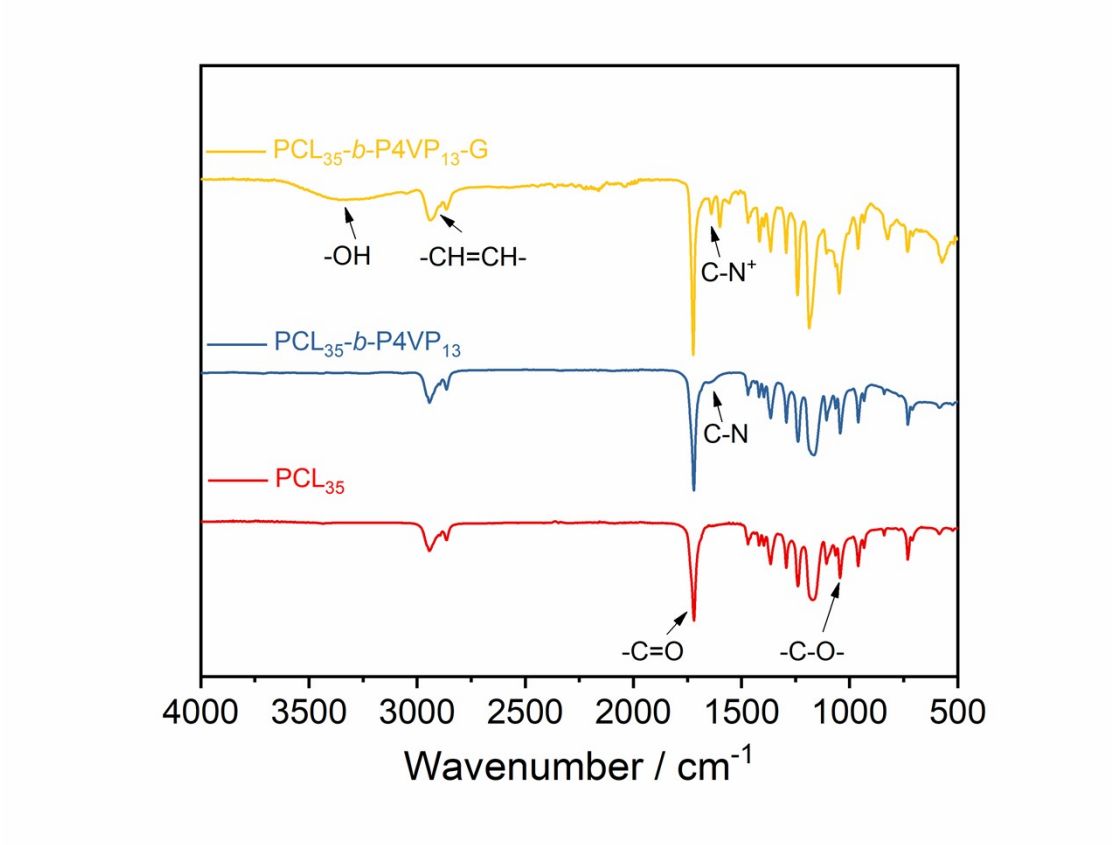


Figure S3. The FTIR spectrogram of PCL_{35}, PCL_{35}-*b*-$P4VP_{13}$, PCL_{35}-*b*-$P4VP_{13}$-G.

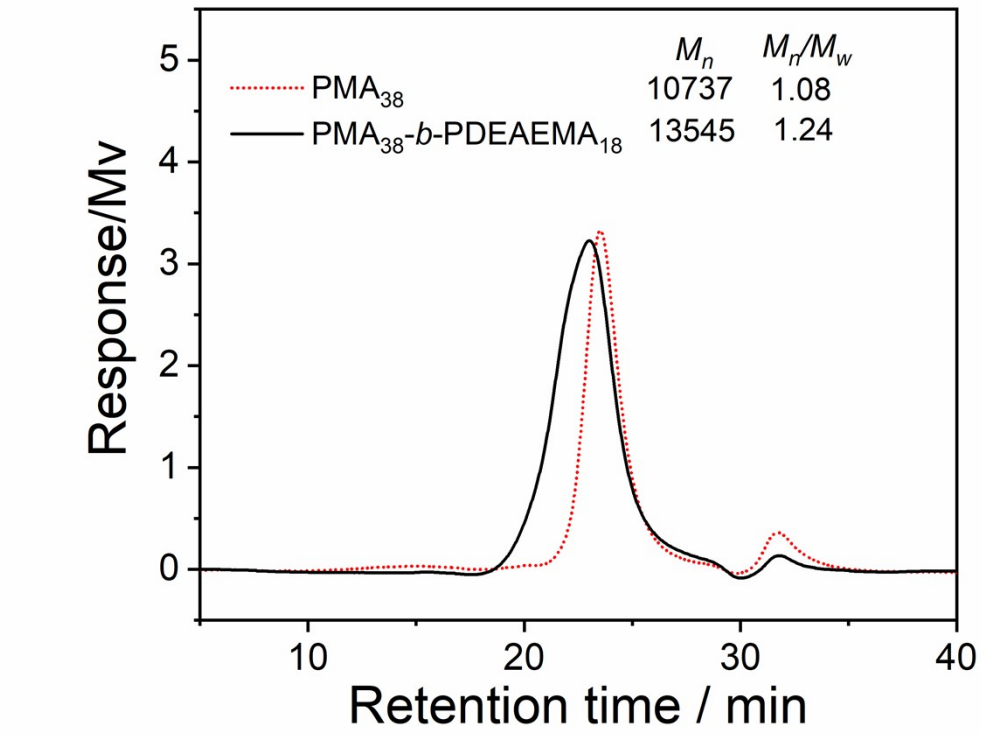


Figure S4. The GPC chromatogram of PMA_{38}, and block polymer (PMA_{38}-*b*-$PDMAEMA_{18}$).

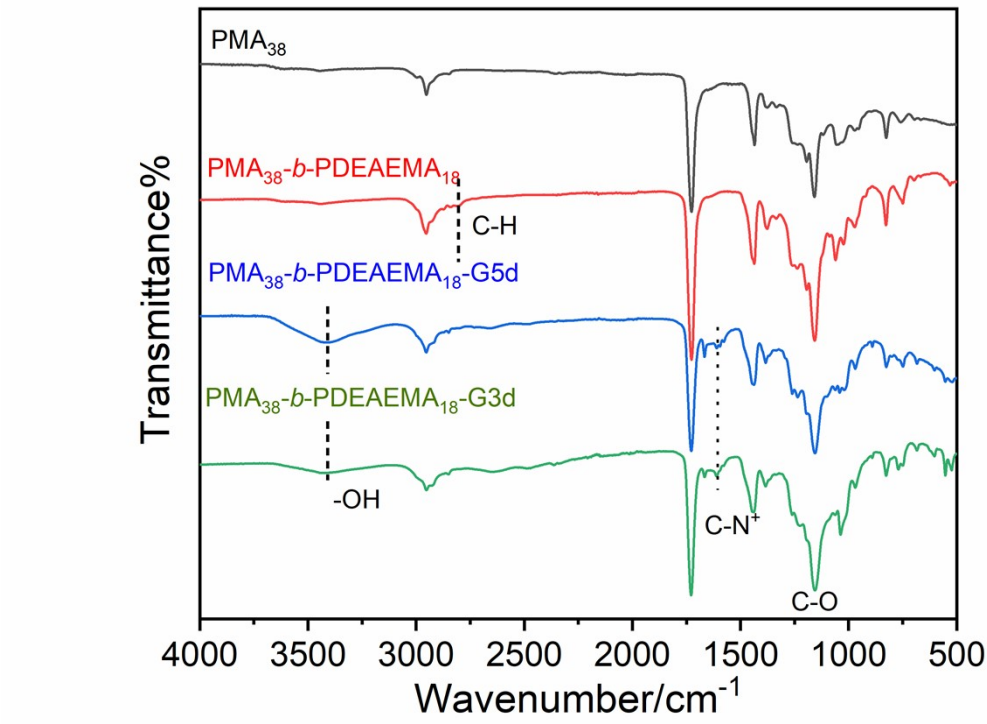


Figure S5. The FTIR spectrogram of PMA_{38}, PMA_{38}-*b*-$PDEAEMA_{18}$, PMA_{38}-*b*-$PDEAEMA_{18}$-G3d, and PMA_{38}-*b*-$PDEAEMA_{18}$-G5d.

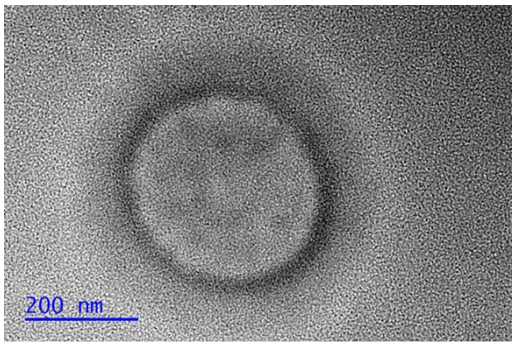
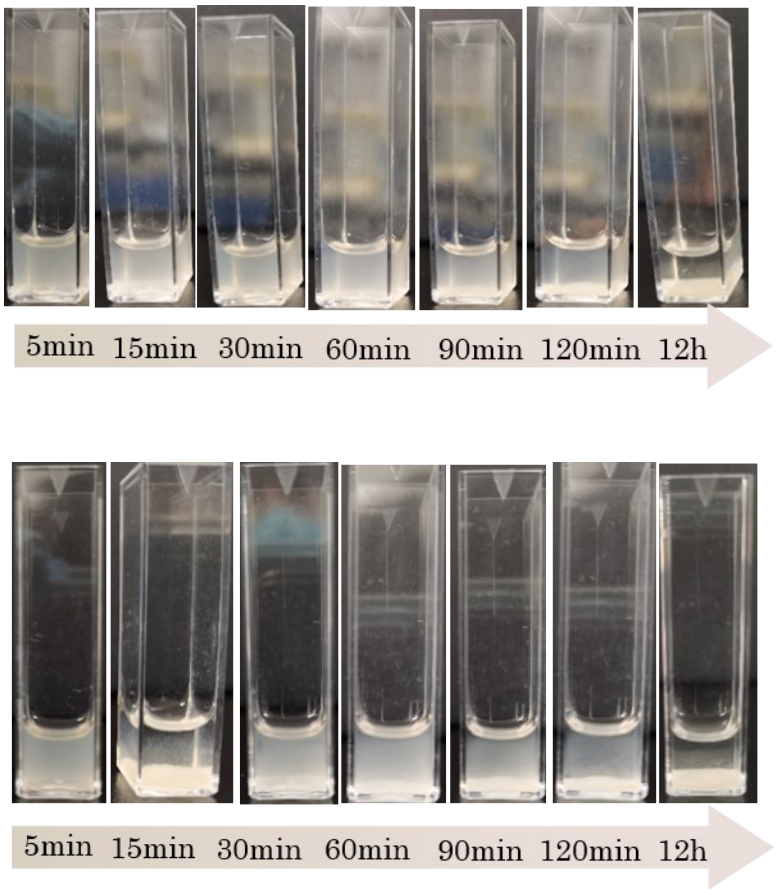


Figure S6. TEM image of glycol-micelles formed by PMA_{38}-*b*-$PDEAEMA_{18}$-G3d.



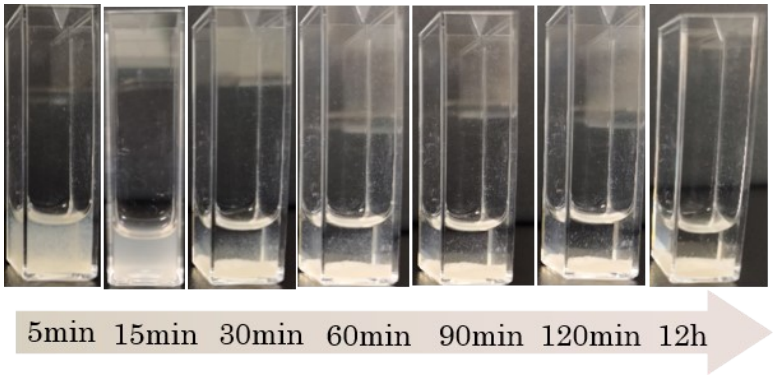


Figure S7. The photograph of state of aggregation for sugar-containing micelle binding with ConA at different times. (These micelles from top to bottom are PCL_{35}-*b*-$P4VP_8$-G, PCL_{35}-*b*-$P4VP_{11}$-G, PCL_{35}-*b*-$P4VP_{13}$-G)

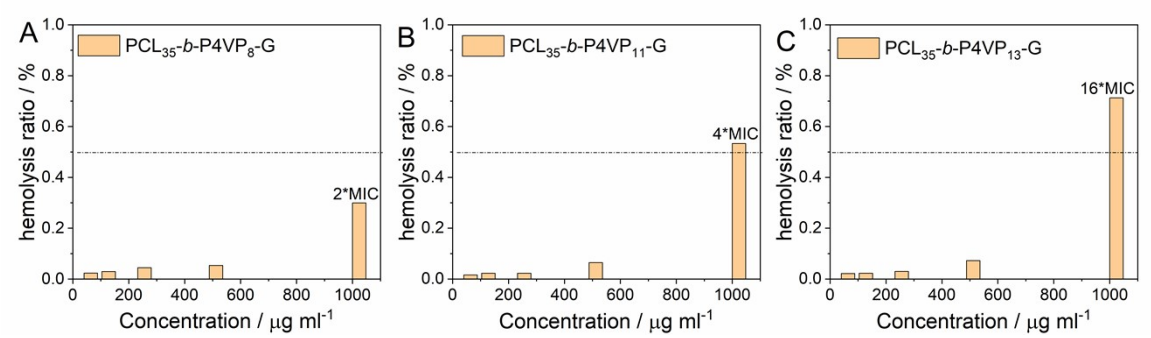


Figure S8. Hemolysis assay of pyridinium-based glycol-micelle against defibriated sheep blood cell. PCL_{35}-*b*-$P4VP_8$-G (A), PCL_{35}-*b*-$P4VP_{11}$-G (B), and PCL_{35}-*b*-$P4VP_{13}$-G (C).

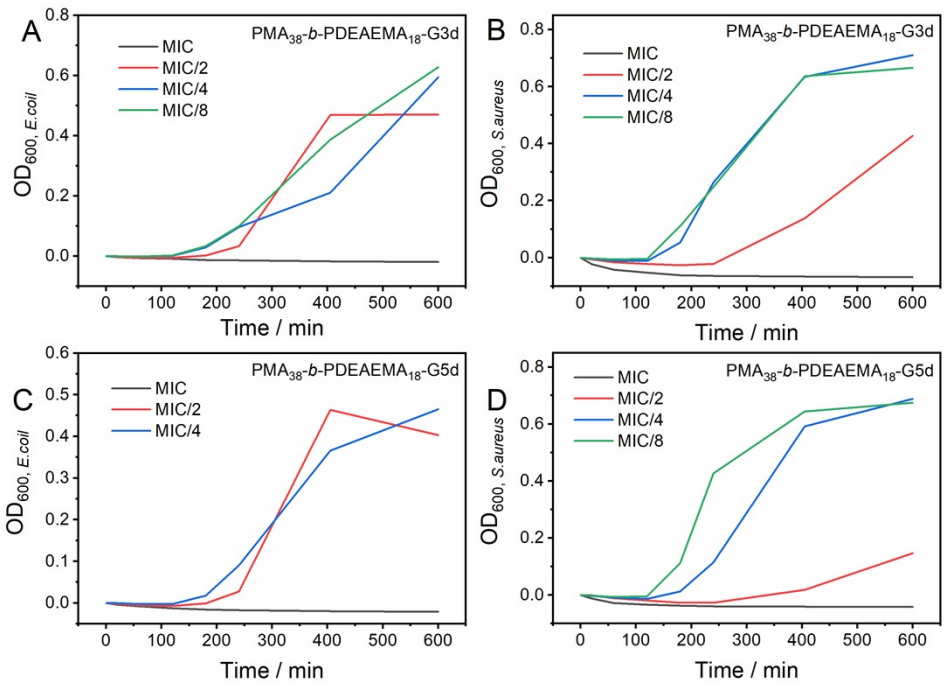


Figure S9. Antimicrobial kinetics on MIC for *E. coli* or *S. aureus* after continuous incubation with different concentrations of quaternary ammonium salt-based glycol-micelles. (A and B: PMA_{38}-*b*-$PDEAEMA_{18}$-G3d, C and D: PMA_{38}-*b*-$PDEAEMA_{18}$-G5d)

Table S1. Molecular weight and molecular weight distribution of pyridinium-based precursor polymers synthesized by changing the mixing ratio of monomer to macromolecular initiator.

	Polymer	CL/VP	$M_{n,\ NMR}$[b]	$M_{n,GPC}$[a]	$M_{w,GPC}$[b]	PDI	ratio
0	PCL_{35}	--	4000	11000	13500	1.16	--
1	PCL_{35}-*b*-$P4VP_8$	35:8	5100	13400	16200	1.21	10:3
2	PCL_{35}-*b*-$P4VP_{11}$	35:11	5400	15200	20000	1.32	10:6
3	PCL_{35}-*b*-$P4VP_{13}$	35:13	5600	16200	29400	1.82	10:9

a Determined by ^{1}H NMR. b Determined by SEC against PMMA standards.

Table S2. Molecular weight and molecular weight distribution of quaternary ammonium salt-based precursor synthesized by changing the ratio of two monomers.

Polymer	MA/DMAEMA	$M_{n,\ NMR}$[b]	$M_{n,GPC}$[a]	$M_{w,GPC}$[b]	PDI	ratio
PMA	--	3300	10700	11600	1.08	--
PMA_{38}-*b*-$PDMAEMA_{18}$	38:18	6100	13500	16800	1.24	10:3

a Determined by ^{1}H NMR. b Determined by SEC against PMMA standards.

Table S3. Summary of particle size, PDI, and zeta-potential of the quaternary ammonium salt-based glycol-assemblies in water.

Polymer	CMC(μg/mL)	Size (nm)	PDI	ξ
PMA_{38}-*b*-$PDEAEMA_{18}$-G	48.0	237.8	0.29	55.1

Table S4. Significantly different in the statistical analysis.

		PCL_{35}-*b*-$P4VP_8$-G	PCL_{35}-*b*-$P4VP_{11}$-G	PCL_{35}-*b*-$P4VP_{13}$-G
MIC	correlation coefficient	0.936*	0.925*	0.931*
	p value	0.019	0.024	0.022

* $p<0.05$ ** $p<0.01$

Reference:

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