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Fast-Bactericidal Effect of Polyion Complex Nanoparticles on Gram-Negative Bacteria

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ABSTRACT

There is urgent need for effective bactericidal agents for use in real commercial formulations since many old disinfectants, such as halogenated compounds, are now banned. Cationic polymers may have good bactericidal properties in pure water or buffer, but typically become ineffective in the presence of anionic surfactants that are widely used in many commercial formulations. Here, we discover that polyion complex (PIC) nanoparticles formed by cationic polymers of Poly(3-AcrylamidoPropyl) TrimethylAmmonium chloride (PAMPTMA) in the presence of anionic surfactants display promising fast-bactericidal effect (> 99.99 % killing within 10-min treatment) on Gram-negative *Escherichia coli* (*E. coli*, ATCC[®] 8739[™]). To examine the influence of hydrophobicity on bactericidal property, we synthesize PAMPTMA-*b*-Poly(Butyl MethAcrylate) and discover that increasing hydrophobicity has little influence on bactericidal property of PIC nanoparticles. Mechanism study shows that cationic PIC nanoparticles rapidly cause significant pores both in the outer- and inner- membranes because of their large size and high local concentration of positive charges. The rapid membrane pore formation results in fast cell death. The discovery -- certain cationic polymers when formulated with anionic surfactants are even more bactericidal than neat cationic polymers alone -- paves the way for potential applications of synthetic cationic polymers in commercial formulations.

1. INTRODUCTION

Infectious disease is a leading cause of death worldwide and accounts for approximately 15 million deaths annually.¹ Antibiotics in use to treat Gram-negative bacterial infections are mostly old drugs discovered more than 5 decades ago.^{2,3} Recent efforts toward discovery of efficient antibiotics have not been productive and are expensive. Meanwhile, bacteria have developed resistance towards all known classes of antibiotics so that AntiMicrobial Resistance (AMR) is now a global healthcare crisis.⁴

Disinfectants and antiseptics have a role to play in addressing this crisis. Antiseptic handwash typically contains halogenated active ingredients such as triclocarban, triclosan, iodophors, *etc.* However, recently, the United States Food and Drug Administration (FDA) has prohibited the use of 19 active ingredients (including triclocarban, triclosan, iodophors, *etc.*) in over-the-counter consumer antiseptic wash products, including hand soap.⁵ Alternative active antiseptic agents which can replace the banned agents and which exhibit comparable or superior efficacy are urgently needed.

One promising class of compounds to combat bacteria is the family of cationic quaternary ammonium polymers (which are frequently called QUATS)⁶⁻⁹, which possesses more potent bactericidal activity than most small molecules such as chelating agents and essential oils. Further, it is well-known that these membrane-active cationic polymers typically lead to negligible evolution of resistance.¹⁰⁻¹² We have recently reported that cationic nanoparticles self-assembled from chitosan-*graft-oligolysine*⁷ or dextran-derived block copolymers⁹ exhibit excellent antibacterial/antibiofilm activity against multidrug-resistant bacteria. We also invented a novel cationic beta-peptide block copolymer that can eradicate all sub-populations of Methicillin-resistant *Staphylococcus aureus* (MRSA).¹³ Recently, Lam *et al.* discovered a class of star-shaped cationic peptide polymer nanoparticles that exerts superior antibacterial activity against a wide range of important Gram-negative bacteria.¹⁰ However, most of these cationic polymers typically would not have bactericidal

effect in commercial formulations containing anionic surfactants/ingredients, such as detergents or personal care products.

In this study, we found that PIC nanoparticles formulated with cationic Poly(3-AcrylamidoPropyl) TrimethylAmmonium chloride (PAMPTMA) (**Scheme 1**) in the presence of anionic surfactants exhibit superior fast-bactericidal effect on *E. coli* (a common Gram-negative bacterium), while individual component of cationic polymers or anionic surfactants alone does not have such effect. We introduced Poly(Butyl MethAcrylate) (PBuMAc), one popular hydrophobic block often used for RAFT polymerization,^{14–17} into PAMPTMA in examining the influence of hydrophobicity on antibacterial activity. Homopolymer PAMPTMA and block copolymer PAMPTMA-*b*-PBuMAc form PIC nanoparticles with various anionic surfactants investigated, such as Sodium Dodecyl Benzene Sulfonate (SDBS), Sodium Dodecyl Sulfate (SDS), Sodium Lauryl Sulfate (SLS), and DiOctyl Sulfosuccinate Sodium salt (DOSS). These cationic PIC nanoparticles kill greater than 99.99 % *E. coli* ($> 4 \log_{10}$ reduction) within 10-min treatment, while poor fast-bactericidal activity was observed with either anionic surfactants or polycations alone. Light scattering measurement reveal that PIC nanoparticles with diameter of about 130 nm formed immediately when polycations were added into anionic surfactants. When formulated with an equal mass amount of anionic surfactants (100 ppm, which is equivalent to 0.1 mg/mL), PIC nanoparticles possess net positive charges which enable them to adhere to *E. coli* surface with electrostatic attraction. With the large size, PIC nanoparticles present a high local concentration of positive charges that facilitate pore formation in both the outer- and inner- membranes. The high propensity of PIC nanoparticles to induce pores may account for their fast-bactericidal effect, which differs dramatically from the mode of action of either anionic surfactants or polycations alone. Anionic surfactant alone cannot penetrate *E. coli* outer membrane while neat polycations only cause a small amount of pores in both the outer- and inner- membranes, which results in much slower bacterial kill.

Scheme 1. Chemical structures of PAMPTMA and PAMPTMA-*b*-PBuMAc (the values of “ n ” and “ m ” are the actual repeat unit numbers).

2. EXPERIMENTAL SECTION

2.1. Materials. All chemicals were used as received unless otherwise indicated. 4-cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl] pentanoic acid (RAFT agent) was acquired from Sigma-Aldrich. 4,4'-Azobis (4-CyanoValeric Acid) (ACVA) was provided by Fluka. Aqueous solution of monomer (3-AcrylaMidoPropyl) TriMethyl-Ammonium chloride (AMPTMA, Sigma-Aldrich) was extracted thrice with diethyl ether to remove inhibitor. Butyl MethAcrylate (BuMAc, Sigma-Aldrich) was de-inhibited by passing the monomers through a column filled with basic alumina prior to reaction. All aqueous solutions were prepared with Milli-Q deionized water with a resistance of 18 M Ω ·cm.

2.2. Synthesis and Characterization of Cationic Polymers. *2.2.1. Synthesis of PAMPTMA.* For the synthesis of homopolymer PAMPTMA (**Scheme 1, Figure S1**), monomer AMPTMA, RAFT agent, initiator ACVA and solvent were combined as listed in **Table S1**. The synthesis of, for example, PAMPTMA₆₅ was performed at a mole feed ratio of [AMPTMA]₀/[RAFT agent]₀/[ACVA]₀ = 100/1/0.2. In a 50 mL round bottom flash equipped with a stirring bar, AMPTMA (6.88 g, 33.3 mmol), RAFT agent (134.6 mg, 0.333 mmol), and ACVA (18.7 mg, 0.0667 mmol) were dissolved in 33 mL of a 7/3 volume mixture of methanol and acetate buffer (pH 5.2). The solution was purged with nitrogen gas for 30 min and then sealed with a rubber stopper. The flask was carried out at 70 °C for 24 hours with stirring. The resulting polymer was purified by dialysis against methanol (1 day) and

water (3 days) with 2000 MWCO dialysis tubing followed by lyophilisation. The cationic polymer PAMPTMA₆₅ was obtained with 65% conversion of the feed AMPTMA monomer. The polymer was characterized carefully, and the results are shown in **Table S2**.

2.2.2. Synthesis of PAMPTMA-*b*-PBuMAc. For the synthesis of block copolymer PAMPTMA-*b*-PBuMAc (**Scheme 1, Figure S1**), monomer (BuMAc), macro RAFT agent (PAMPTMA₁₈₃), initiator (ACVA) and solvent (methanol) were combined as listed in **Table S1**. Typically, PAMPTMA₁₈₃-*b*-PBuMAc₄ was synthesized at feed ratio of [BuMAc]₀/[macro RAFT agent PAMPTMA₁₈₃]/[ACVA]₀ = 10/1/0.2. BuMAc (25 mg, 0.172 mmol), PAMPTMA₁₈₃ (657 mg, 0.0172 mmol), ACVA (1 mg, 0.00357 mmol) and methanol (1 mL) were placed in a 10 mL round bottom flask equipped with a stirring bar. The flask was purged with nitrogen gas for 15 min and then sealed with a rubber stopper. The reaction was carried out at 65 °C for 8 hours with stirring. Upon completion, the reaction was quenched in an ice bath and one aliquot was sampled for ¹H NMR analysis. The monomer conversion was determined by ¹H NMR analysis. The polymer was purified via dialysis against methanol (2 days). After removal of solvent and drying under vacuum, polymer PAMPTMA₁₈₃-*b*-PBuMAc₄ was characterized by ¹H NMR and GPC (**Table S2**).

2.2.3. ¹H NMR. Proton Nuclear Magnetic Resonance (¹H NMR) spectra were obtained with an AVANCE 300 MHz spectrometer (Bruker, U.S.A), using the solvent signal for calibration (**Figure S2-S5**).

2.2.4. GPC. The molecular weight and dispersity were measured using a Waters' Gel Permeation Chromatography (GPC) system equipped with a 2410 Refractive Index Detector (RID), using two ultra-hydrogel columns and sodium acetate buffer (0.5 M of sodium acetate and 0.5 M of acetic acid, pH ~ 4.5) as mobile phase at 40 °C with a flow rate of 1.0 mL/min. Monodisperse pullulan was used as the standard to generate the calibration curve (**Figure S6**).

2.3. Antibacterial Assay. **2.3.1. Culture of *E. coli*.** *E. coli*, ATCC® 8739™, was purchased from American Type Culture Collection (ATCC). To prepare a culture for the antibacterial assay, *E.*

coli frozen stock culture was streaked on a Lysogeny Broth (LB) agar (Difco) plate and the plate was incubated overnight at 37 °C. A single, well-isolated colony was picked from the plate and sub-cultured on a LB agar plate. This *E. coli* was used in the antibacterial assay.

2.3.2. Detergent Formulation. Commercial liquid detergent, containing 10 wt% of anionic surfactants, was provided by Procter & Gamble Company. In this study, 10 wt% SDBS (Sigma-Aldrich) aqueous solution was used to mimic the commercial liquid detergent.

2.3.3. Quench Solution. 42.8 g of modified Lethen broth (Difco), 10 g of Tween 80 (Sigma-Aldrich), and 9.3 g of soy lecithin (Wako) were added to 1 L of water. The mixture was stirred until all the solid had dissolved. The quench solution was sterilised by autoclaving prior to use.

2.3.4. Time-kill Suspension Test. European suspension test protocol¹⁸ was employed to measure antibacterial activity of cationic polymers in this study. First, 10 mg/mL of cationic polymers and 1 mg/mL of SDBS were prepared separately in water as stock solution. Then as shown in **Scheme 2**, 1 mL of SDBS stock solution was mixed with 8 mL of water prior the addition of 0.1 mL of cationic polymer stock solution. After vortex for 1 min, 1 mL of *E. coli* suspension in 0.85 wt% sodium chloride (O.D.₆₀₀ = 0.12, corresponding to ~ 10⁸ CFU/mL) was added to the test formulation and incubated at room temperature for 10 min. The antibacterial assay was terminated by 10-fold dilution of assay mixture in quench solution. After quenching the antibacterial assay, six 10-fold serial dilutions in 0.85 wt% sodium chloride were performed, and quintuplicate samples (5 µL) were spread on LB agar plate. The number of individual colonies on each plate was counted after overnight incubation at 37 °C.

Scheme 2. Schematic representation of PIC nanoparticle preparation and time-kill suspension test.

2.4. Laser Light Scattering. Malvern Nano Series, Nano-ZS, equipped with a 532 nm laser, was used to determine the hydrodynamic radius (R_h) of cationic polymers in water and in SDBS. All the data was collected with the instrument-standard backscattering detector (173°) at 25°C . Radius of gyration (R_g) was measured with a Brookhaven BI-200SM equipped with 637 nm laser. All experiments were done at room temperature ($\approx 22^\circ\text{C}$). A 2 mm pinhole before the photomultiplier tube detector was used to adjust light scattering intensity. A Guinier plot, $\ln I = \ln I_0 - \frac{q^2 R_g^2}{3}$, was used to estimate R_g , where I is scattered light intensity, I_0 is the scattered light intensity at $q \rightarrow 0$, and q is scattering wave vector.¹⁹ For light scattering measurement, PIC nanoparticles were prepared by adding 0.1 mL of polycation stock solution (10 mg/mL) and 1 mL of SDBS stock solution (1 mg/mL) into 9 mL of water. PIC nanoparticles were measured directly without membrane filtration.

2.5. Zeta-Potential Measurement. Zeta-potential measurement was performed with a Malvern Nano Series, Nano-ZS. Pure polycation and SDBS aqueous solution were prepared separately by diluting each stock solution to final concentration of 100 ppm. PIC nanoparticles were prepared per the procedure in the light scattering measurement. Overnight *E. coli* cultures were washed thrice with 0.85 wt% sodium chloride, and diluted to an O.D.₆₀₀ value of 0.12, and used immediately. The

value of zeta-potential was measured three times (each measurement being the average of 10 runs) and the mean values and standard deviations were calculated.

2.6. Nitrocefin Hydrolysis Assay. Nitrocefin (*i*-DNA Biotechnology Pte Ltd) was used as the probe to measure outer membrane permeabilization. 5 mg/mL of nitrocefin stock solution was prepared with DMSO as the solvent. This stock solution was then diluted to 0.2 mg/mL with sterile water.²⁰ *E. coli* from single-colony subculture was diluted to O.D.₆₀₀ = 0.12 with 5 mM of glucose (Fluka) aqueous solution containing 1 μ M of Carbonyl Cyanide *m*-ChloroPhenylhydrazone (CCCP, Sigma-Aldrich). Then, 140 μ L of *E. coli* suspension was pipetted into the well of a 96-well plate followed by the addition of bactericidal agents to make up a total volume of 180 μ L. After 10-min treatment, 20 μ L of nitrocefin solution (0.2 mg/mL) was added immediately and nitrocefin cleavage was recorded for 1 hour by light absorption measurement at 500 nm (PerkinElmer LS-55 luminescence spectrometer). The final concentration of bactericidal agents in 200 μ L solution was kept the same as in time-kill suspension test (100 ppm). At the end of the absorption measurement, the visual appearance of the cultures was recorded with a digital camera.

2.7. DiSC3-5 Assay. *E. coli* from single-colony subculture was suspended in water to an O.D.₆₀₀ = 0.12. 1 mL of 10-times diluted cell suspension was placed in a 1 cm cuvette followed by the addition of EDTA solution to attain final concentration of 0.2 μ M. The fluorescence reading was monitored at an excitation wavelength of 622 nm and an emission wavelength of 670 nm. After 10-min treatment with EDTA, 3,3'-dipropylthia-dicarbocyanine iodide (diSC3-5) dye was added to attain a final concentration of 0.4 μ M. The fluorescence was recorded until diSC3-5 uptake was maximal (as indicated by a stable reduction in fluorescence due to fluorescence quenching as the dye became concentrated in the cell inner membrane).²¹ Then bactericidal agent was added and the fluorescence increase due to membrane potential dissipation was recorded. The final concentration of bactericidal agents was kept the same as in the time-kill suspension test (100 ppm).

2.8. Flow Cytometry. *E. coli* from single-colony subculture was treated with bactericidal agents following the same protocol used in the time-kill suspension test. After 10-min treatment, cells were harvested by centrifugation at 6000 rpm for 3 min, then resuspended in 0.5 mL of water. The cell suspension was incubated with Propidium Iodide (PI, final concentration was 3 μ M) for 15 min before flow cytometry measurement (using FL-2 channel).

2.9. Liposome Preparation. Lipid stock solutions (10 mg/mL) of POPC (1-Palmitoyl-2-Oleoyl-*sn*-glycero-3-PhosphoCholine), and POPG (1-Palmitoyl-2-Oleoyl-*sn*-glycero-3-Phospho-(1'-*rac*-Glycerol), sodium salt) were prepared in methanol/chloroform (3:1, *v/v*). 10 mg of LPS (LipoPolySaccharide from *E. coli* O111:B4) was dispersed in 2 mL of methanol/chloroform (3:1, *v/v*) and sonicated at room temperature for 20 min. LPS solution (5 mg/mL) looked quite hazy and faint yellow. Mixture of POPC-LPS (4:1, *m/m*) was prepared by mixing 0.8 mL of POPC stock solution with 0.4 mL of LPS stock solution in a round-bottomed flask (10 mL). Organic solvent was evaporated with rotary evaporation, then the lipid film was hydrated with 1 mL of water. The suspension was then vortexed for 2 min at top speed followed by 2 min sonication at room temperature. This vortex-sonication process was performed 3 times, and the solution was then extruded 15 times through 200 nm polycarbonate membrane with Avanti mini-extruder. The size distribution and zeta-potential of the resulting liposomes were checked with Malvern Nano Series, Nano-ZS (**Table S3**). POPC-POPG (4:1, *m/m*) liposome was prepared by the same procedure. The prepared liposome solutions were stored at 4 °C prior to use.

2.10. Isothermal Titration Calorimetry (ITC). Calorimetric measurement was conducted using a PEAQ-ITC from MicroCal Malvern. We prepared liposomes (1 mg/mL) and different titrants, such as 100 ppm of SDBS, 100 ppm of PAMPTMA₃₁₀, and PIC nanoparticles (100 ppm of SDBS + 100 ppm of PAMPTMA₃₁₀) in water. 1 μ L of titrant was added via syringe to liposome solution in the cell at 200 s intervals with 750 rpm stirring speed. 30 injections were performed in each experiment. The temperature was kept at 25 °C with reference power set at 10 μ cal/s. The dilution heat was

recorded by injecting 1 μL of titrants into water and was subtracted from the corresponding integrated injection heat.

3. RESULTS AND DISCUSSION

We first synthesized a series of PAMPTMA homopolymer with different molecular weights via RAFT polymerization (**Scheme 1**). The number of AMPTMA repeat units was successfully controlled by varying the monomer/RAFT agent ratio (**Table S1**). Block copolymer PAMPTMA-*b*-PBuMAc was synthesized using PAMPTMA₁₈₃, *i.e.*, poly(AMPTMA) with 183 actual repeat units of AMPTMA, as a macro RAFT agent (**Table S1**). ^1H NMR spectra (**Figure S2-S5**) demonstrate the successful preparation of PAMPTMA and PAMPTMA-*b*-PBuMAc in a controlled way and the number of actual repeat units was quite close to the design value (**Table S4**). Using ^1H NMR and GPC (**Figure S6**), the measured number-average molecular weights, *i.e.*, $M_{n,1\text{H NMR}}$ and $M_{n,\text{GPC}}$, and the dispersity index (PDI_{GPC}) indicate that the polymerization is well controlled (**Table S2**).

Polymer antibacterial activity is determined by a short-duration (10 min) time-kill suspension test according to the European suspension test protocol.^{18,22} All the polycations exhibit poor fast-bactericidal activities in pure water with $\log_{10}$ reductions < 1.0 (**Table 1**). However, all the polymers show remarkable fast-bactericidal activities in the presence of either SDBS or a commercial liquid detergent. In 10-min exposure to the mixture of 100 ppm polycations and 100 ppm SDBS (or commercial liquid detergent containing 100 ppm of anionic surfactants), *E. coli* is killed with, typically, $> 5 \log_{10}$ reduction in the presence of SDBS and $> 4 \log_{10}$ reduction in the presence of liquid detergent. Similar fast-bactericidal activities were observed for the mixtures of our polycations with other anionic surfactants, specifically SDS, SLS and DOSS (**Table S5**). Increasing polycation concentration dramatically improves the bactericidal activity (**Table S6**). We observed that *E. coli* is eradicated within 10 min ($> 8 \log_{10}$ reduction) when polycations reach 200 ppm in the presence of 100 ppm of SDBS, indicating that antibacterial activity appears to depend on the amount of positive charges

in formulations. Such observation is consistent with the work of Leticia *et al.*²³ From the previous reports,^{24,25} we expected that adding hydrophobicity would increase polymer antibacterial activity. Instead, the results suggest that introducing PBuMAc block and increasing its block length do not influence polymer antibacterial activity either in water or in anionic surfactants (**Table 1**). Generally, high hydrophobicity would facilitate the insertion of polycations into the hydrophobic core of cell membranes and cause more effective membrane disruption,²⁶ while excessive hydrophobicity would cause aggregation and tend to block membrane penetration.²⁷ Poly(AEVE)-*b*-poly(IBVE), which possesses a similar structure to PAMPTMA-*b*-PBuMAc, displayed a decreased bactericidal activity with longer hydrophobic poly(IBVE) blocks.²⁸ Kenichi and co-workers have revealed that antimicrobial activity changes sigmoidally with the mole fraction of hydrophobic groups (f_{HB}).²⁴ As f_{HB} increases, antimicrobial activity starts low, increases sharply, and then plateaus. In our study, f_{HB} (calculated as m/n) of PAMPTMA-*b*-PBuMAc is 1.6% ($m = 4$ and $n = 183$) and 14% ($m = 26$ and $n = 183$) respectively. Such low value of f_{HB} could result in a similar antibacterial activity as homopolymers. Great degree of toxicity (**Figure S7**) and poor antimicrobial activity against *S. aureus* 29213 and *Candida albicans* 10231 (**Table S7**) were observed for PIC nanoparticles.

The enhanced antibacterial activity of PAMPTMA and PAMPTMA-*b*-PBuMAc in the presence of anionic surfactants is an exception to the well-established principle that, because of charge neutralization, anionic surfactants reduce the antibacterial activity of cationic polymers.²⁹ To understand this unusual antibacterial activity displayed by AMPTMA-derived polycations in the presence of anionic surfactants, we studied the particle formation and interaction forces when these cationic polymers were mixed with anionic surfactants.

Table 1. Bactericidal activities against *E. coli* in 10-min treatment.

Antibacterial agents (100 ppm)	Log ₁₀ reduction ^a		
	In water	In SDBS (100 ppm)	In detergent (100 ppm) ^b
SDBS	0.2 ± 0.1	0.3 ± 0.1	0.1 ± 0.1
PAMPTMA ₆₅	0.6 ± 0.1	5.8 ± 0.8	3.9 ± 1.1
PAMPTMA ₁₈₃	0.5 ± 0.1	5.5 ± 1.0	5.4 ± 1.4
PAMPTMA ₃₁₀ ^c	0.6 ± 0.1	6.8 ± 0.9	5.9 ± 1.2
PAMPTMA ₁₈₃ - <i>b</i> -PBuMAc ₄	0.5 ± 0.1	5.1 ± 1.1	6.2 ± 2.0
PAMPTMA ₁₈₃ - <i>b</i> -PBuMAc ₂₆	0.5 ± 0.1	6.2 ± 1.4	5.3 ± 1.8

^a the value is an average of three independent measurements with uncertainties (±) estimated by one standard deviation of the mean. ^b the commercial liquid detergent contains 100 ppm of anionic surfactants. ^c Toxicity (**Figure S7**) and antimicrobial activity against *S. aureus* 29213 and *Candida albicans* 10231 (**Table S7**) were measured.

In water, no reliable light-scattering signal was observed for polycations at 100 ppm (**Table 2**), indicating that polycations do not form stable aggregates at such low concentration. Neat SDBS at 100 ppm exists as unimers in water since its Critical Micelle Concentration (*CMC*) is measured as 127 ppm at room temperature with the fluorescent pyrene probe method³⁰ (**Figure S8**). Our *CMC* of 127 ppm corroborates the reported *CMC* of 131 ppm for SDBS at 25 °C.³¹ A slightly cloudy solution was observed when adding 100 ppm polycations into 100 ppm SDBS (the same turbidity was also observed with all the other anionic surfactants investigated). DLS measurement indicates that PIC nanoparticles form immediately when adding polycations into anionic surfactants (**Figure S9**). We observed that SDBS mixing with PAMPTMAs with varying molecular weights form PIC nanoparticles with an almost constant, *i.e.*, independent of homopolymer chain length, hydrodynamic radius R_h of ~ 67 nm (**Table 2**). R_h increases slightly with the addition of hydrophobic block PBuMAc₄ to PAMPTMA (R_h = 74 nm), while it doubles with a longer block of PBuMAc₂₆ (R_h = 134 nm). With the low dispersity (PDI) observed in all cases (**Table 2**), we suggest that the complex structure of PIC nanoparticles is well-equilibrated. To determine the shape of PIC nanoparticles, we also measured the radius of gyration R_g with static light scattering. R_g of PIC nanoparticles prepared from homopolymer PAMPTMA remains virtually the same (R_g ~ 110 nm), while R_g of those prepared from block copolymers increases with PBuMAc block length (**Table 2**). For all PIC nanoparticles, independent

of the molecular weight, the value of R_g/R_h is about 1.7. Such high R_g/R_h value indicates that PIC nanoparticles could possess an anisotropic shape.³²

It appears that zeta-potential of homopolymers PAMPTMA trend more positive with increasing chain length, while block copolymers PAMPTMA-*b*-PBuMAc present even higher zeta-potential than homopolymers. It is known that zeta-potential significantly depends on particle property and solution conditions.^{33–35} In our work, deionized water is used for all the DLS and zeta-potential measurements, therefore there is no difference in solution conditions (such as ionic strength and composition). For homopolymers PAMPTMA, as chain length increases, the number of positive charges (quaternary ammonium groups) contained in each polymer chain increases. The higher number of positive charges could result in a higher zeta-potential at longer chain length. For block copolymer PAMPTMA-*b*-PBuMAc, transient association/assembly could exist. These transient association/assembly may not produce reliable signal in DLS, but could possess higher amount of positive charges, thus leading to a higher zeta-potential value. We observed that all PIC nanoparticles are highly positively charged, in spite of the presence of anionic surfactants (**Table 2**). The positive zeta-potential is due to > 1.0 ratio of moles of cationic charges (of the polymer at 100 ppm) to moles of anionic charges (due to surfactants present at 100 ppm) (**Table S8**).

We measured R_h and zeta-potential of PIC nanoparticles formulated with different polycation concentrations (**Table S9**). The results suggest that R_h of PIC nanoparticle (in the range of 60 to 90 nm) displays no clear dependence on polymer length, polymer composition and polymer concentration. In water, zeta-potential of PAMPTMA₆₅ and PAMPTMA₁₈₃ increases with polymer concentration, while zeta-potential of PAMPTMA₃₁₀, and two block copolymers fluctuates randomly with polymer concentration. In SDBS, zeta-potential of PIC nanoparticles somehow increases with polycation concentration, which could be attributed to the high amount of positive charges concentrated in PIC nanoparticles.

Table 2. Structure characteristics obtained with light scattering and zeta-potential measurements.

Samples (100ppm)	In water ^a		In SDBS (100 ppm) ^a			
	R_h [nm] ^b	zeta-potential [mV]	R_h [nm] (PDI) ^c	R_g [nm] ^c	R_g/R_h	zeta-potential [mV]
PAMPTMA ₆₅	n/a	+34.6 ± 3.5	67 ± 0.2 (0.16)	116 ± 0.3	1.7 ± 0.01	+38.3 ± 0.4
PAMPTMA ₁₈₃	n/a	+39.2 ± 3.9	67 ± 0.1 (0.15)	108 ± 1.1	1.6 ± 0.02	+42.4 ± 1.1
PAMPTMA ₃₁₀	n/a	+42.7 ± 2.5	64 ± 0.4 (0.18)	114 ± 0.2	1.8 ± 0.01	+43.0 ± 0.5
PAMPTMA ₁₈₃ - <i>b</i> -PBuMAC ₄	n/a	+52.6 ± 2.5	74 ± 0.2 (0.19)	145 ± 1.0	1.9 ± 0.01	+43.1 ± 0.5
PAMPTMA ₁₈₃ - <i>b</i> -PBuMAC ₂₆	n/a	+51.1 ± 1.3	134 ± 1.6 (0.31)	168 ± 0.3	1.3 ± 0.02	+43.0 ± 0.3
SDBS	n/a	-35.2 ± 2.3				
<i>E. coli</i> (O.D. ₆₀₀ = 0.12)	620	-42.0 ± 0.6				

^a the value is an average of three independent measurements with uncertainties (±) estimated by one standard deviation of the mean. ^b n/a: no reliable light-scattering signal was observed for polycations (100 ppm) and SDBS (100 ppm). ^c original correlation function and R_h distribution, and Guinier plot for R_g estimation were provided in **Figure S10** and **S11**.

We probed the permeabilization of bacterial Outer Membrane (OM) using nitrocefin hydrolysis assay. Nitrocefin, a chromogenic β -lactam, is normally excluded by bacterial OM. However, if the OM is permeabilized so that the molecule is able to cross this barrier, nitrocefin can be cleaved by β -lactamase localized within the periplasmic space, resulting in a color change from yellow to red.³⁶ We probed the possible color changes for (#1) neat PAMPTMA₃₁₀, (#2) neat SDBS and (#3) PIC nanoparticles (the mixture of SDBS with PAMPTMA₃₁₀). *E. coli* was treated with different solutions for 10 min and then the nitrocefin probe was added. For solution #1 (PAMPTMA₃₁₀) and #3 (PIC nanoparticles), a visual color change from yellow to red was observed (**Figure 1a**, inset). This is corroborated by the sharp absorbance increase at 500 nm (**Figure 1a**), indicating permeabilization of the OM. For solution #2 (SDBS), no color change nor increase in absorbance at 500 nm was observed, indicating that SDBS does not permeabilize the OM (**Figure 1a**). Here, we use nitrocefin-hydrolysis rate (the initial slope upon the addition of nitrocefin in **Figure 1a**) to quantify OM-disruption capability of different solutions. For PIC nanoparticles (#3), SDBS (#2) and PAMPTMA₃₁₀ (#1), the nitrocefin-hydrolysis rate was 6.6×10^{-6} , 2.1×10^{-6} and 3.7×10^{-6} M/min respectively (**Table S10**), indicating that PIC nanoparticles (#3) possess a higher capability of OM disruption than neat SDBS (#2) and neat

polycations (#1). The higher membrane-disruptive capability may be attributed to the larger size ($R_h \approx 70$ nm) of PIC nanoparticles which would cause significant membrane rupture around the contact point.³⁷

Figure 1. (a) OM permeabilization test with nitrocefin after 10-min treatment of *E. coli* with different solutions. The inset shows color changes at the end of the assay. (b) IM potential assay with diSC3-5. *E. coli* was treated with EDTA for 10 min to break the OM prior the addition of dye diSC3-5. (c) Flow cytometry experiment of *E. coli* stained with dye PI. The concentrations of SDBS, PAMPTMA₃₁₀ and polymyxin B were 100 ppm each. (d) Summary of the results of various membrane assays with PAMPTMA₃₁₀, SDBS, and PIC nanoparticles. Action capability: - (no effect), + (weak), ++ (medium), +++ (strong).

We then probed the Inner Membrane (IM) potential change using diSC3-5 dye, which is typically taken up by live cells which have an IM potential gradient and then accumulates in the cell membrane leading to self-quenching of its own fluorescence.²¹ Permeabilization of the IM by bactericidal agent would collapse the potential gradient and result in a release of diSC3-5 from cells to solution, with a consequent increase in fluorescence. In **Figure 1b**, intact *E. coli* was pre-treated with EDTA to increase the OM permeability prior to the addition of diSC3-5 so that dye molecules could readily reach the IM. Then diSC3-5 dye was added, and after waiting for the dye fluorescence signal to plateau to near zero (after about 40-60 min), agents #1-#3 were each added separately, and the dye fluorescence due to the agent's IM permeabilization was tracked. The fluorescence increase caused by PIC nanoparticles (488 a.u.) is 5.3- and 1.3-fold higher than the respective increases caused by polycations (101 a.u.) and SDBS (370 a.u.) (**Figure 1b**). The ability of the respective agents to alter bacterial IM potential gradient is ranked as follows: polycations < SDBS < PIC nanoparticles. SDBS is slightly less effective than PIC nanoparticles at collapsing the IM potential gradient and both are significantly more effective than polycations alone.

We also used PI dye, which stains chromosomes and thus is a useful fluorescent probe for cell death due to the permeabilized OM and IM.³⁸ Once bound to DNA, PI fluorescence intensity increases, which may be employed in flow cytometry experiment to count dead cells with permeable membrane. In **Figure 1c**, compared with untreated cells, cells treated with SDBS (#2) and PAMPTMA₃₁₀ (#1) exhibit only a small peak attributable to dead cells (the “shoulder” on the high-fluorescence side of the “live cells” peak in #1 and #2). These results suggest that both SDBS (#2) and polycations (#1) disrupt the membranes of only a small fraction of the cells challenged with these agents (consistent with the low log₁₀ reductions in pure water, **Table 1**). As summarized in **Figure 1d**, SDBS (#2) cannot disrupt the OM (**Figure 1a**) but can disturb the IM potential gradient if the OM has been “opened” by EDTA (**Figure 1b**), but the PI assay indicates DNA staining of a small fraction of the SDBS-treated cells (**Figure 1c**), implying that SDBS by itself cannot eradicate the bacteria (consistent with **Table 1**)

because of the OM barrier. Polycations employed alone (#1) can perturb the OM (**Figure 1a**) and also disrupt the IM (**Figure 1b**) albeit only to a limited extent compared to PIC nanoparticles (#3), so that polycations produce a weak but more pronounced PI signal compared to SDBS (**Figure 1c**). The rapid (10-min exposure) killing efficacy of neat polycations is poor (**Table 1**) but with longer exposure (24h), all neat polycations eradicate *E. coli* with log₁₀ reductions above 8 (**Figure S12**). Different to neat SDBS and neat polycations, PIC nanoparticles produce a large high-fluorescence peak in PI signal, *i.e.*, due to permeabilized/dead cells, and reduced the “live cell” fluorescence signal to an undetectable level (**Figure 1c**). We infer that PIC nanoparticles can significantly alter and form pores in both the OM and IM in essentially all exposed cells (**Figure 1a, b**) so that there is substantial PI entry into virtually all the exposed cells (**Figure 1c**). PIC nanoparticles (#3) can also effectively fast-kill bacteria, with log₁₀ reduction of several in 10 min (**Table 1**). Taken together, these studies indicate that PIC nanoparticles have high potency to rapidly induce pores in both the OM and IM and produce fast-bactericidal effect on *E. coli* cells (**Figure 1d**).

We employed ITC to further study the PIC nanoparticle-membrane interaction. It has been established that ITC can be used to elucidate the threshold for pore formation and micellization.³⁹ The OM and IM of Gram-negative *E. coli* were respectively modeled with mixtures of POPC:LPS (4:1, *m/m*) and POPC:POPG (4:1, *m/m*). **Figure 2** shows the ITC traces measured during injection of different titrants into POPC-LPS or POPC-POPG liposomes. For SDBS titrating into POPC-LPS (**Figure 2a**) and POPC-POPG (**Figure 2b**), each injection produces an exothermic heat that is virtually independent of SDBS concentration. The exothermic heat produced in these experiments is probably related to the transfer of aqueous SDBS unimers into lipid bilayers. Drastically different ITC traces were observed for PAMPTMA₃₁₀ and PIC nanoparticles (**Figure 2c-f**). PAMPTMA₃₁₀ interacting with liposomes (**Figure 2c, d**) displays an initial increase of the exothermic heat with the number of injections, which is then followed by a decrease of the exothermic heat. This inverted bell-shaped thermodynamic profile is the evidence of membrane pore formation.³⁹ We suggest that PAMPTMA₃₁₀

chains adhere to liposomes driven by electrostatic attraction of the positively charged polymer to the negatively charged liposome outer surface. PAMPTMA₃₁₀ interacts locally with anionic lipids leading to lipid extraction from the lipid bilayer and finally to pore formation.^{37,40} For both liposomes, a second exothermic peak appears at titrant/lipid ≈ 0.027 and 0.022 respectively, a behavior which has been proposed to coincide with the formation of polycation-lipid aggregates.³⁹ Pore formation was also observed in the titration of liposomes with PIC nanoparticles (formed with mixing 100 ppm SDBS and 100 ppm PAMPTMA₃₁₀) (**Figure 2e, f**). The pore formation energy ΔQ_{pore} is calculated according to previous studies (**Figure S12**).^{39,41} ΔQ_{pore} of PIC nanoparticles and PAMPTMA₃₁₀ titrated into POPC-POPG liposomes is -4.4 and -0.86 kJ/mol respectively (**Figure S12a**). For POPC-LPS liposomes, ΔQ_{pore} produced by PIC nanoparticles and PAMPTMA₃₁₀ is -4.6 and -3.0 kJ/mol respectively (**Figure S12b**). The larger pore formation energy caused by PIC nanoparticles suggests a higher pore density in lipid bilayers, which may be attributable to the high concentration of positive charges in PIC nanoparticles.

Figure 2. ITC calorimetric heat traces (Differential Power, DP) and the integrated heat of injection (ΔQ). (a, c, e) POPC-LPS liposome, mimicking the OM of *E. coli*, was titrated with SDBS, PAMPTMA₃₁₀, and (SDBS+PAMPTMA₃₁₀) respectively. (b, d, f) POPC-POPG liposome, mimicking the IM of *E. coli*, was titrated with SDBS, PAMPTMA₃₁₀, and (SDBS+PAMPTMA₃₁₀) respectively.

Based on the above studies, we suggest the bactericidal mechanism shown in **Scheme 3**. *E. coli* Gram-negative cell wall has an IM, a thin peptidoglycan layer, and an OM covered with LPS in its outer leaflet⁴² (**Scheme 3a**). *E. coli* cell surface is highly negatively charged ($\zeta = -42.0$ mV, **Table S11**) while changes to positively charged immediately upon the addition of cationic PIC nanoparticles ($\zeta = +17.9$ mV, **Table S11**), which indicates that PIC nanoparticles attach to *E. coli* cell surface rapidly as a result of electrostatic attraction. The strong attachment of cationic PIC nanoparticles to anionic cell surface likely plays a critical role in their bactericidal activity. Anionic PIC nanoparticles (**Table S12**), formed with 50 ppm polycations in commercial liquid detergent containing 100 ppm anionic

surfactants, exhibit poor bactericidal activity against *E. coli*, probably due to their poor attachment to the anionic cell surface. Similar behavior was reported that negatively charged cells are 100 % viable while positively charged cells do not survive.⁴³ A clear correlation was observed between cell death and attachment of cationic liposomes generating positively charged cell surface.⁴³ It is therefore necessary that polycation concentration be sufficiently high to produce high amount of positive charges in PIC nanoparticles (in most of the experiments in this paper, in the presence of 100 ppm anionic surfactant, 100 ppm polycation appears to be sufficient). PIC nanoparticles possess a higher amount of positive charges exhibit better killing effect (**Table S6**). Once PIC nanoparticles attach to cell surface, severe OM distortion at the contact point would happen rapidly. We have previously reported that cationic hydrogel can kill contacted bacteria by acting as “anion sponges” to “suction” anionic lipids from bacterial membrane into the hydrogel interior.⁴⁴ The large size ($R_h \approx 70$ nm, of order 10 % of the target cell size) and high positive surface charges ($\zeta \approx +40$ mV) enable PIC nanoparticles to strongly perturb negatively charged membrane components (LPS and anionic lipids) and induce pores rapidly. The presence of large numbers of surfactants within PIC nanoparticles may promote solvation of membrane constituents into the PIC interior; alternatively or additionally, surfactants may migrate from the PIC nanoparticles into the IM to permeabilize it. Collectively, nitrocefin and diSC3-5 assays, flow cytometry and ITC provided evidence for OM rupture, IM potential gradient dissipation, and pore formation in both the OM and IM. Taken together, these studies suggest that PIC nanoparticles rapidly cause cell death through pore formation by disrupting the OM and IM, as depicted in **Scheme 3d**.

Scheme 3. (a) Intact *E. coli* envelope structure. (b-d) Proposed action of mechanism for *E. coli* treated with SDBS, polycations, and PIC nanoparticles in 10-min treatment. The relative sizes of the representations of the various species are not proportional to the actual size (in particular, the PIC nanoparticles are much larger than depicted; roughly 10 % of the size of the targeted cell).

In contrast, the mechanisms of individual polycation or SDBS acting on *E. coli* are probably completely different. We shall first discuss the mechanism of polycations. Previous study has revealed that polycations could bind to LPS exterior via electrostatic attraction and competitively displace the divalent cations that partially neutralize LPS negative charges⁴⁵. Our experiments show that zeta-potential of *E. coli* changes dramatically upon the addition of polycations (**Table S11**). Moreover, nitrocefin hydrolysis assay indicates that polycations cause severe damage (pore formation) in OM (**Figure 1a**). Nanopore formation and bulges in OM induced by antimicrobial polycations has been analyzed with real-time atomic force microscopy.⁴⁶ Polycations of dimethylaminoethyl-chitosan has been demonstrated to bind to LPS by electrostatic interactions, thus disrupting the integrity of the

OM.⁴⁷ Similarly, in our study polycations could disrupt the physical integrity of OM via pore formation,¹⁰ but are mostly trapped by electrostatic interaction with the LPS of OM (**Scheme 3c**) so that with brief exposure they only minimally alter the IM permeability (**Figure 1b**). In short exposure time (10 min), neat polycation causes no IM pores as measured by the PI assay (**Figure 1c**) and results in only modest consequences for cell death (**Table 1**). While with longer exposure, enhanced bactericidal activity was observed that all *E. coli* is eradicated ($\log_{10}$ reduction > 8) above 12 hours exposure (**Figure S13**). The difference in the mode of action between linear polycations and PIC nanoparticles could be attributed to their different sizes, as large PIC nanoparticles would concentrate positive charges and act as “mobile anion sponge” to accelerate membrane deformation/disruption that leads to fast (within 10 min) cell death (**Scheme 3d**).

Unlike polycations and PIC nanoparticles, SDBS is negatively charged ($\zeta = -35$ mV, **Table 2**). ITC studies indicate that SDBS interacts with lipid bilayers (**Figure 2a, b**), but it cannot penetrate the OM (**Figure 1a**) and hence it cannot reach the IM and hence cannot lead to cell death (**Table 1**). SDBS hardly induces membrane pore formation (**Figure 1c, Scheme 3b**). It should be noted that though **Scheme 3** is proposed based on our best understanding of current experimental data (**Figure 1 and 2**), the details are still speculative and needed further studies.

To examine whether the fast-bactericidal effect is a universal property of cationic PIC nanoparticles, we tested several other cationic polymers, and found that PolyDiAllylDiMethylAmmonium Chloride (PDADMAC) and PolyMETformin (PMET) show good antibacterial activity in water but reduced killing effect in SDBS (**Table S13**). Branched PolyEthylenImine (BPEI) exhibits poor activity in both water and SDBS. Polylysine and PolyAllylamine Hydrochloride (PAH) show poor activity in water but enhanced activity in SDBS. It seems that linear polycations containing moderate alkyl groups within their monomers, such as polylysine, PAH and AMPTMA-derived polymers reported here, display fast-bactericidal effect in the presence of anionic surfactants. On the other hand, poor fast-bactericidal effect was observed for PIC

nanoparticles prepared from PDADMAC, PMET, and BPEI which are made from monomers that do not have pendant alkyl groups. Based on these observations, we conclude that pendant alkyl groups are required in polycations to enable fast-bactericidal activity of PIC nanoparticles. To test the effect of counterions, we replaced monovalent anions SDBS with multivalent anions Poly(4-StyreneSulfonic acid) sodium salt (PSS), and found that PIC nanoparticles formulated with 100 ppm of polycation in 100 ppm of PSS (**Figure S14**) show poor fast-bactericidal activity (**Figure S14**), though these PIC nanoparticles are positively charged ($R_h = 52$ nm, $\zeta = 36.9$ mV). Correlation between counterion and antimicrobial activity of polycations was reported before.⁴⁸ Tew and co-workers expected to use hydrophobic counterions to tune polycations hydrophobicity and thus antimicrobial activity with less synthetic effort.⁴⁹ While the results were unfortunate because hydrophobic counterions form tight ion pairs with ammonium groups and mask overall positive charges, thus results in a loss of membrane activity and antimicrobial activity of polycations.⁴⁹ In our study, PSS-polycation pair possesses a much lower bactericidal activity than SDBS-polycation pair, which indicates that monovalent counterion could be another requirement for fast-bactericidal activity besides pendant alkyl groups in polycations and enough positive charges in PIC nanoparticles.

4. CONCLUSIONS

In summary, cationic polymers PAMPTMA and PAMPTMA-*b*-PBuMAc formulated with anionic surfactants exhibit superior fast-bactericidal activity against Gram-negative *E. coli*, 99.99% killing within 10-min treatment, compared to either cationic polymer or anionic surfactant alone, which is not bactericidal on such short time scale. This is contrary to many other findings that cationic polymers lost their bactericidal activity because of fouling by anionic surfactants and is explained by the formation of cationic PIC nanoparticles. The cationic PIC nanoparticles concentrate positive charges and attach to anionic bacteria surface, resulting in a high local concentration of positive charges to rapidly cause significant permeabilization of both the OM and IM, leading to fast cell death.

This successful demonstration of fast-bactericidal activity of PIC nanoparticles against Gram-negative bacteria has myriad potential applications in many commercial formulations *e.g.* consumer product formulations, for which alternative antibacterial agents are urgently needed. This novel approach to fast-acting chemical antiseptics with surfactant formulations may also be useful in clinical medical settings, which tend to be contaminated with antibiotic-resistant pathogens.

Supporting Information

Synthesis schematic of cationic polymers; polymerization conditions of cationic polymers; characteristics of cationic polymers; ^1H NMR spectrum of PAMPTMA₆₅; ^1H NMR spectrum of PAMPTMA₁₈₃; ^1H NMR spectrum of PAMPTMA₃₁₀; ^1H NMR spectrum of PAMPTMA₁₈₃-*b*-PBuMAc₄; water GPC of PAMPTMA homopolymer; characteristics of liposomes; comparison of design and actual repeat unit numbers; antibacterial activity of cationic polymers in different surfactants; bactericidal activity of polycations as a function of polymer concentration; MTT assay with 3T3 cells; antibacterial performance of PIC nanoparticles against Gram-positive bacteria and fungus; *CMC* measurement of SDBS; time-dependence of correlation function and hydrodynamic diameter; calculated molar ratios of cationic to anionic charges in PIC nanoparticles; PIC nanoparticle size and zeta-potential as a function of polycation concentration; original data of correlation function and the corresponding R_h distribution; Guinier plot for R_g estimation; calculation of nitrocefin hydrolysis rate; determination of pore formation energy ΔQ_{pore} ; zeta-potential changes of *E. coli* as a function of time during treatment; zeta-potential and bactericidal activity of (50 ppm polycation + 100 ppm surfactant); time-kill suspension test at different contact times; antibacterial activity of commercial polycations; killing effect of PAMPTMA₃₁₀ in the presence of PSS; DLS measurement of PIC nanoparticles formed with PAMPTMA₃₁₀ and PSS.

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Fast-bactericidal effect is exhibited with polyion complex (PIC) nanoparticles formed by polycations and anionic surfactants. Unlike polycations and anionic surfactants alone, cationic PIC nanoparticles kill > 99.99 % *E. coli* within 10-min treatment. This study paves the way for potential applications of polycations in liquid detergent formulation and complex media.

Supporting Information

Fast-Bactericidal Effect of Polyion Complex Nanoparticles on Gram-Negative Bacteria

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Figure S1-Synthesis schematic of cationic polymers

Table S1-Polymerization conditions of cationic polymers

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Figure S3- ^1H NMR spectrum of PAMPTMA₁₈₃

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Figure S1. Schematic of preparation of homopolymer PAMPTMA and block copolymer PAMPTMA-*b*-PBuMAc.

Table S1. Polymerization conditions of homopolymer PAMPTMA and block copolymer PAMPTMA-*b*-PBuMAc.

No.	Polymers	[M]/[RAFT]/[ACVA]	Solvent	Yields
1a	PAMPTMA ₆₅	100/1/0.2	Methanol/Acetate buffer (7/3 v/v)	65 %
1b	PAMPTMA ₁₈₃	300/1/0.2	Methanol/Acetate buffer (7/3 v/v)	61 %
1c	PAMPTMA ₃₁₀	500/1/0.2	Methanol/Acetate buffer (7/3 v/v)	62 %
2a	PAMPTMA ₁₈₃ - <i>b</i> -PBuMAc ₄	10/1/0.2	Methanol	40 %
2b	PAMPTMA ₁₈₃ - <i>b</i> -PBuMAc ₂₆	50/1/0.2	Methanol	52 %

Table S2. Polymer characteristics of homopolymer PAMPTMA and block copolymer PAMPTMA-*b*-PBuMAc.

No.	Polymers	M_n , $^1\text{H NMR}$ [g/mol]	M_n , GPC [g/mol]	PDI _{GPC}
1a	PAMPTMA ₆₅	17400	21758	1.10
1b	PAMPTMA ₁₈₃	38200	59635	1.10
1c	PAMPTMA ₃₁₀	64500	80958	1.21
2a	PAMPTMA ₁₈₃ - <i>b</i> -PBuMAc ₄	38700	-	-
2b	PAMPTMA ₁₈₃ - <i>b</i> -PBuMAc ₂₆	41900	-	-

Figure S2. ^1H NMR spectrum of PAMPTMA₆₅ in D_2O . The extra peak A could derive from alkene protons of unreacted AMPTMA monomers, and extra peak B could derive from CH_2CO group in RAFT agent.^{1,2}

Figure S3. ^1H NMR spectrum of PAMPTMA₁₈₃ in D₂O. The extra peak A could derive from alkene protons of unreacted AMPTMA monomers.^{1,2}

Figure S4. ^1H NMR spectrum of PAMPTMA₃₁₀ in D₂O. The extra peak A could derive from alkene protons of unreacted AMPTMA monomers.^{1,2}

Figure S5. ¹H NMR spectrum of PAMPTMA₁₈₃-*b*-PBuMAc₄ in D₂O.

Figure S6. Water GPC of PAMPTMA homopolymer.

Table S3. Characteristics of liposomes.

Liposomes	R_h [nm] (PDI)	Zeta-potential [mV]
POPC-LPS	63 (0.17)	-17.3 ± 0.3
POPC-POPG	63 (0.14)	-43.7 ± 1.0

Table S4. Comparison of design and actual repeat unit numbers of cationic polymers.

Polymers	Design values		Actual values	
	n	m	n	m
PAMPTMA _{n}	100	-	65	-
PAMPTMA _{n}	300	-	183	-
PAMPTMA _{n}	500	-	310	-
PAMPTMA _{n} - b -PBuMAc _{m}	300	10	183	4
PAMPTMA _{n} - b -PBuMAc _{m}	300	50	183	26

Table S5. Antibacterial activity of cationic polymers in Sodium Dodecyl Sulfate (SDS), Sodium Lauryl Sulfate (SLS), and DiOctyl Sulfosuccinate Sodium salt (DOSS). The contact time is 10 min.

Cationic polymer at 100 ppm	$\log_{10}$ reduction		
	In 100 ppm of SDS	In 100 ppm of SLS	In 100 ppm of DOSS
PAMPTMA ₆₅	8.8	2.2	4.5
PAMPTMA ₁₈₃	8.8	4.2	8.7
PAMPTMA ₃₁₀	8.8	4.5	4.8
PAMPTMA ₁₈₃ - b -PBuMAc ₄	8.8	4.4	8.7

Table S6. Bactericidal activity of polycations as a function of polymer concentration. The contact time is 10 min.

Polycations	Polycation concentration [ppm]	Log ₁₀ reduction	
		In water	In SDBS (100 ppm)
PAMPTMA ₆₅	50	0.48 ± 0.05	1.63 ± 0.50
	100	0.58 ± 0.07	5.81 ± 0.83
	200	0.61 ± 0.05	8.64 ± 0.11
PAMPTMA ₁₈₃	50	0.40 ± 0.08	3.17 ± 1.25
	100	0.52 ± 0.07	5.53 ± 0.98
	200	0.63 ± 0.08	8.67 ± 0.13
PAMPTMA ₃₁₀	50	0.62 ± 0.10	3.02 ± 0.48
	100	0.67 ± 0.06	6.78 ± 0.79
	200	0.75 ± 0.10	8.26 ± 0.46
PAMPTMA ₁₈₃ - <i>b</i> -PBuMAc ₄	50	0.39 ± 0.12	4.02 ± 1.58
	100	0.48 ± 0.10	5.12 ± 1.10
	200	0.68 ± 0.04	8.58 ± 0.28
PAMPTMA ₁₈₃ - <i>b</i> -PBuMAc ₂₆	50	0.39 ± 0.06	4.08 ± 1.57
	100	0.46 ± 0.11	6.23 ± 1.36
	200	0.65 ± 0.05	8.58 ± 0.28

Figure S7. MTT assay with 3T3 cells (3×10^5 cells/mL). Great degree of toxicity was observed for 100 ppm of SDBS, 100 ppm of polycations (PAMPTMA₃₁₀) and PIC nanoparticles (100 ppm of SDBS + 100 ppm of PAMPTMA₃₁₀).

Table S7. Antibacterial performance of PIC nanoparticles against Gram-positive bacteria (*S. aureus* 29213) and fungus (*Candida albicans* 10231). The contact time is 10 min. The cell concentration is 4×10^8 and 1.3×10^8 CFU/mL for *S. aureus* and *Candida albicans* respectively. PIC nanoparticles cannot kill Gram-positive and fungus as effective as *E. coli*.

	Log ₁₀ reduction	
	PAMPTMA ₃₁₀ (100 ppm) in water	PIC nanoparticles (100 ppm of PAMPTMA ₃₁₀ + SDBS 100ppm)
Gram-positive (<i>S. aureus</i> 29213)	0.09	0.09
Fungus (<i>Candida albicans</i> 10231)	0.00 ± 0.01	0.03 ± 0.02

Figure S8. (a) The emission spectrum of pyrene in SDBS over the concentration range 5 to 10^4 ppm. (b) Plot of the intensity ratio I_3/I_1 versus $\log c$. The solid line represents the fit by a Boltzmann-type sigmoid. The Critical Micelle Concentration (CMC) obtained was 127 ppm, in good agreement with previous studies.³

Figure S9. Time-dependence of (a) correlation function and (b) hydrodynamic diameter of PIC nanoparticle formed with polycation (100 ppm of PAMPTMA₃₁₀) and anionic surfactant (100 ppm of SDBS).

Table S8. Calculated molar ratios of cationic to anionic charge in PIC nanoparticles formed with SDBS.

Polymers	Cationic charges (+) for 100 ppm of polymer [M]	Anionic charges (-) for 100 ppm of SDBS [M]	Molar ratio (+/-)
PAMPTMA ₆₅	4.85×10^{-4}	2.87×10^{-4}	1.69
PAMPTMA ₁₈₃	4.85×10^{-4}	2.87×10^{-4}	1.69
PAMPTMA ₃₁₀	4.85×10^{-4}	2.87×10^{-4}	1.69
PAMPTMA ₁₈₃ - <i>b</i> -PBuMAC ₄	4.73×10^{-4}	2.87×10^{-4}	1.65
PAMPTMA ₁₈₃ - <i>b</i> -PBuMAC ₂₆	4.37×10^{-4}	2.87×10^{-4}	1.52

Table S9. PIC nanoparticle size and zeta-potential as a function of polycation concentration.

Polycations	Polycation concentration [ppm]	R_h of PIC nanoparticles [nm]	Zeta-potential	
			In water	In SDBS (100 ppm)
PAMPTMA ₆₅	50	82	28.1 ± 4.5	30.9 ± 0.3
	100	67	34.6 ± 3.5	38.3 ± 0.4
	200	66	41.4 ± 2.8	59.0 ± 0.8
PAMPTMA ₁₈₃	50	61	51.7 ± 2.2	27.9 ± 0.5
	100	67	39.2 ± 3.9	42.4 ± 1.1
	200	65	46.8 ± 2.2	53.5 ± 2.1
PAMPTMA ₃₁₀	50	71	50.1 ± 1.7	29.6 ± 0.2
	100	64	42.7 ± 2.5	43.0 ± 0.5
	200	72	50.6 ± 2.1	38.4 ± 1.4
PAMPTMA ₁₈₃ - <i>b</i> -PBuMAc ₄	50	82	51.3 ± 2.4	33.4 ± 0.7
	100	74	52.6 ± 2.5	43.1 ± 0.5
	200	56		
PAMPTMA ₁₈₃ - <i>b</i> -PBuMAc ₂₆	50	61	52.3 ± 4.3	31.6 ± 0.8
	100	134	51.1 ± 1.3	43.0 ± 0.3
	200	60

Figure S10. Original correlation function (a – e) and the corresponding R_h distribution (f – j) for PIC nanoparticles formulated with 100 ppm of polycations and 100 ppm SDBS.

Figure S11. Guinier plot for R_g estimation for PIC nanoparticles formulated with 100 ppm of polycation and 100 ppm of SDBS.

Table S10. Calculation of nitrocefin hydrolysis rate.

Samples	ΔA [a.u.]	Δt [min]	Δc [M] ^a	Nitrocefin hydrolysis rate [M/min] ^b
#1: PAMPTMA ₃₁₀	0.06	1.4	5.1×10^{-6}	3.7×10^{-6}
#2: SDBS	0.03	1.4	2.8×10^{-6}	2.1×10^{-6}
#3: PIC nanoparticle	0.10	1.4	9.1×10^{-6}	6.6×10^{-6}

^a The change of hydrolyzed-nitrocefin concentration Δc was calculated by the Lambert-Beer law $\Delta A = \varepsilon \cdot l \cdot \Delta c$ where ΔA is the change of absorbance during reaction, ε is the molar extinction coefficient of hydrolyzed-nitrocefin ($17500 \text{ M}^{-1} \cdot \text{cm}^{-1}$), and l is the sample path length measured in experiment (l is about 0.62 cm for 200 μL solution pipette in 96-well plate).

^b Nitrocefin hydrolysis rate was calculated as $\text{rate} = \frac{\Delta c}{\Delta t}$ where Δc and Δt is respectively the change of hydrolyzed-nitrocefin concentration and the change of reaction time.

Figure S12. Accumulated molar heat ($\Delta Q_{acc}/C_{titrant}$) is shown as a function of the molar ratio of titrant/lipid. Accumulated heat (ΔQ_{acc}) was calculated as the total heat accumulated up to injection i , $\Delta Q_{acc} = \sum_{i=1}^n \Delta Q_i$ where ΔQ_i is the integrated heat of each injection.^{4,5} The pore formation energy ΔQ_{pore} was defined as the maximal accumulated molar heat, as shown in the graph.

Table S11. Zeta-potential changes of *E. coli* as a function of time when treated with PAMPTMA₃₁₀ (100 ppm) and PIC nanoparticles (100 ppm SDBS + 100 ppm PAMPTMA₃₁₀).

	Time [min]	Zeta potential of <i>E. coli</i> [mV] ^a
<i>E. coli</i> treated with PAMPTMA ₃₁₀	0-	-42.0 ± 0.6
	0+	+28.5 ± 0.3
	5	+30.8 ± 0.3
	10	+32.1 ± 0.2
<i>E. coli</i> treated with PIC nanoparticles (SDBS+PAMPTMA ₃₁₀)	0-	-42.0 ± 0.6
	0+	+17.9 ± 0.6
	5	+19.0 ± 0.5
	10	+19.7 ± 0.3

^a the value is an average of three independent measurements with uncertainties (±) estimated by one standard deviation of the mean.

Table S12. Zeta-potential values and bactericidal activities against *E. coli* within 10 min for polycations (50 ppm) in aqueous solution of commercial liquid detergent containing 100 ppm of anionic surfactants.

Polymers at 50 ppm	Zeta-potential in detergent (100 ppm) [mV]	Log ₁₀ reduction in detergent (100 ppm)
PAMPTMA ₆₅	-45.9 ± 0.6	0.21 ± 0.04
PAMPTMA ₁₈₃	-47.7 ± 1.0	0.21 ± 0.05
PAMPTMA ₃₁₀	-50.4 ± 0.7	0.22 ± 0.03
PAMPTMA ₁₈₃ - <i>b</i> -PBuMAc ₄	-26.6 ± 0.5	0.22 ± 0.06
PAMPTMA ₁₈₃ - <i>b</i> -PBuMAc ₂₆	-26.6 ± 1.3	0.25 ± 0.05

Figure S13. Time-kill suspension test of 100 ppm polycations against *E. coli* in water.

Table S13. Antibacterial activity of commercial polycations in water and in 100 ppm of SDBS, respectively. The contact time is 10 min.

Antibacterial agents (100 ppm)	Log ₁₀ reduction		Zeta-potential of PIC nanoparticle [mV]	R _h of PIC nanoparticle [nm] (PDI)
	In water	In SDBS (100 ppm)		
PDADMAC _{400k}	8.64	0.07	+20.6 ± 0.4	108 ± 10.1 (0.29)
PMET	1.44	0	-	-
BPEI _{25k}	0.2 ± 0.2	0.8 ± 0.4	+61.3 ± 1.1	104 ± 2.5 (0.03)
PAH _{15k}	0.9	3.27	+70.8 ± 0.2	100 ± 1.2 (0.10)
Poly-L-lysine _{30k}	1.1 ± 1.2	5.6 ± 2.7	+41.7 ± 0.3	76 ± 1.4 (0.20)

PDADMAC is PolyDiAllylDiMethylAmmonium Chloride; PMET is PolyMETformin; PAH is PolyAllylamine Hydrochloride and BPEI is Branched PolyEthylenImine.

Table S14. Killing effect of PAMPTMA₃₁₀ in the presence of multivalent anion of Poly(4-StyreneSulfonic acid) sodium salt (PSS). The contact time is 10 min.

Antibacterial agents (100 ppm)	Log ₁₀ reduction	
	In water	In PSSNa (100 ppm)
PSSNa ($M_w \approx 70$ kDa)	0.19 ± 0.05	0.19 ± 0.05
PAMPTMA ₃₁₀	0.96 ± 0.09	0.75 ± 0.05

Figure S14. DLS measurement of PIC nanoparticles formed with 100 ppm of PAMPTMA₃₁₀ in the presence of 100 ppm of Poly(4-StyreneSulfonic acid) sodium salt (PSS).

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