Supporting information.

Cationic Methacrylate Polymers as Topical Agents against *Staphylococcus aureus* Nasal Colonization

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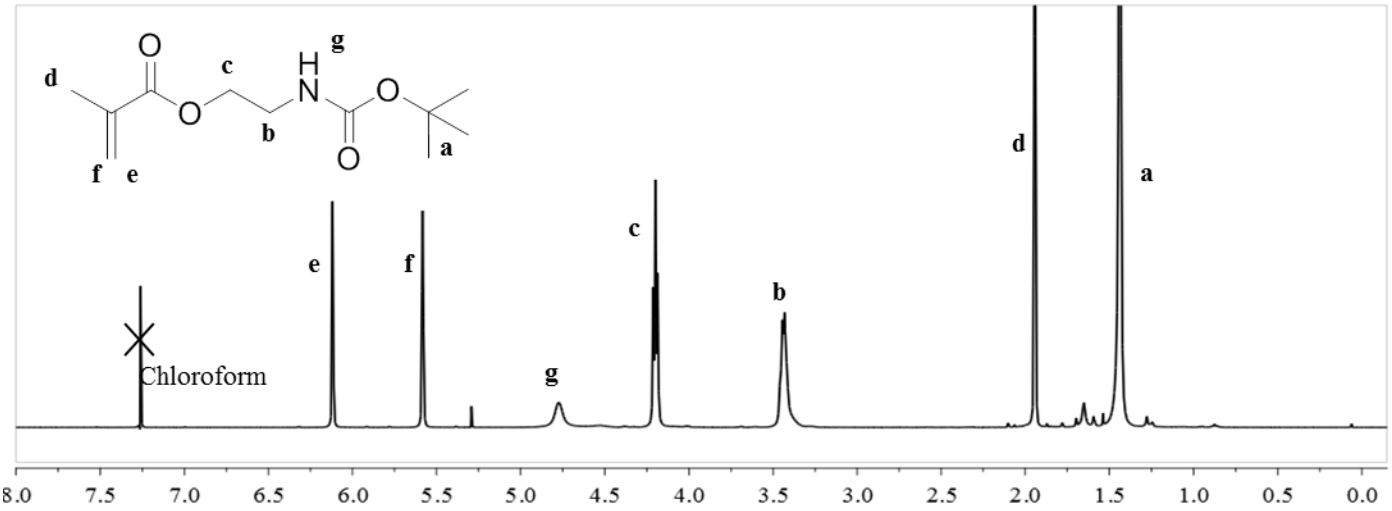
1. ^{1}H NMR spectra of monomer and polymers.

N-(*tert*-butoxycarbonyl)aminoethyl methacrylate (Boc-AEMA) ($CDCl_3$)



2-cyanoprop-2-yl ethyl trithiocarbonate (CPETC) ($CDCl_3$)

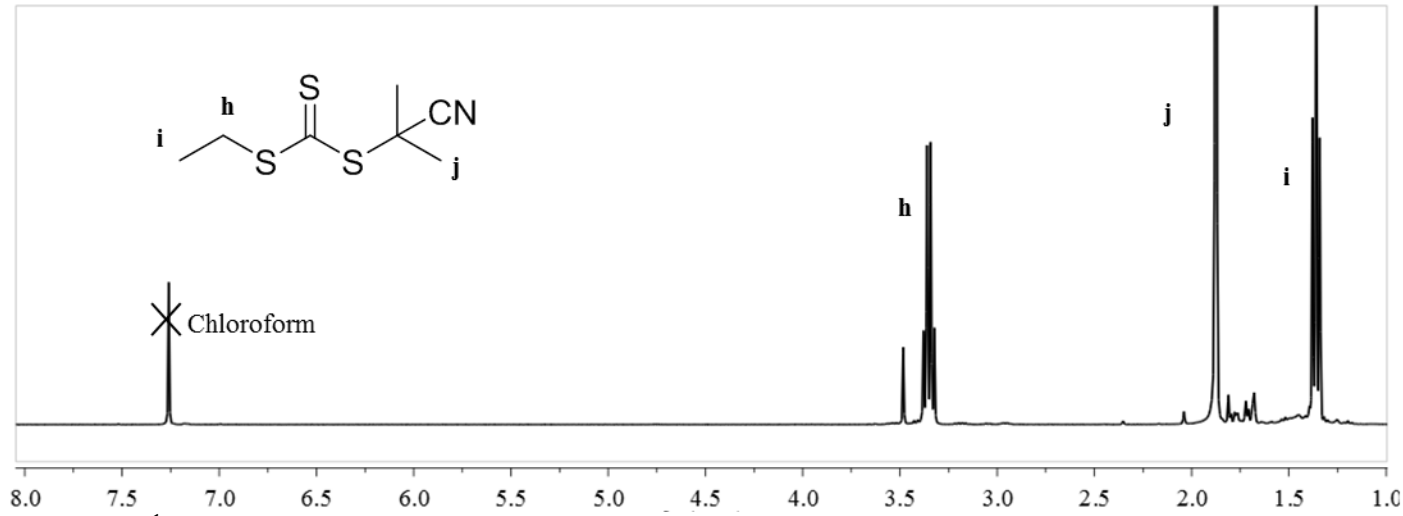
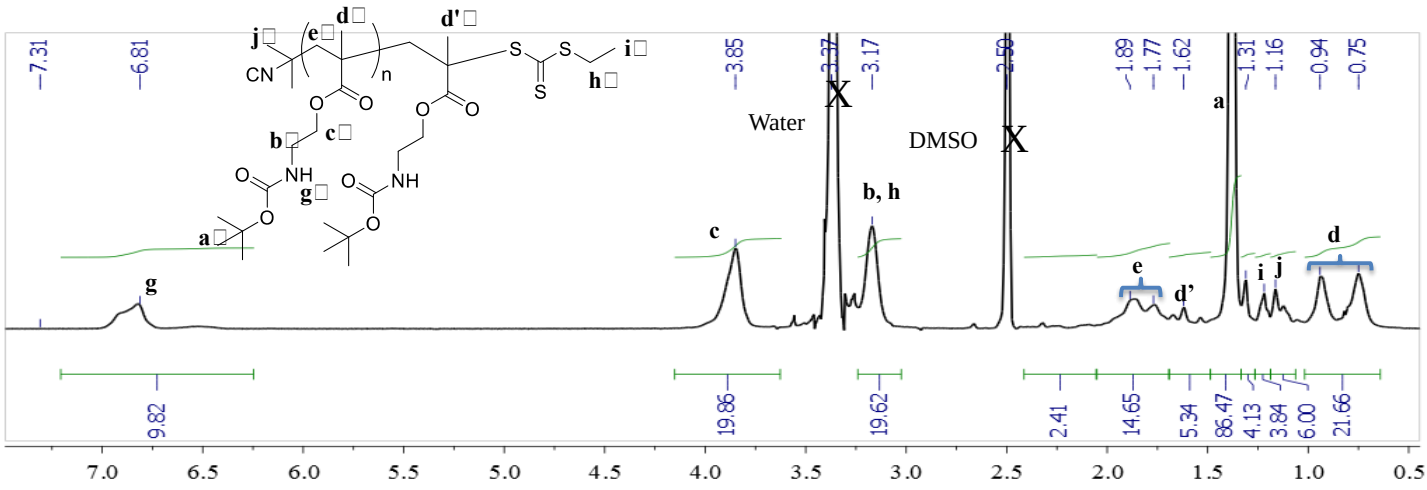
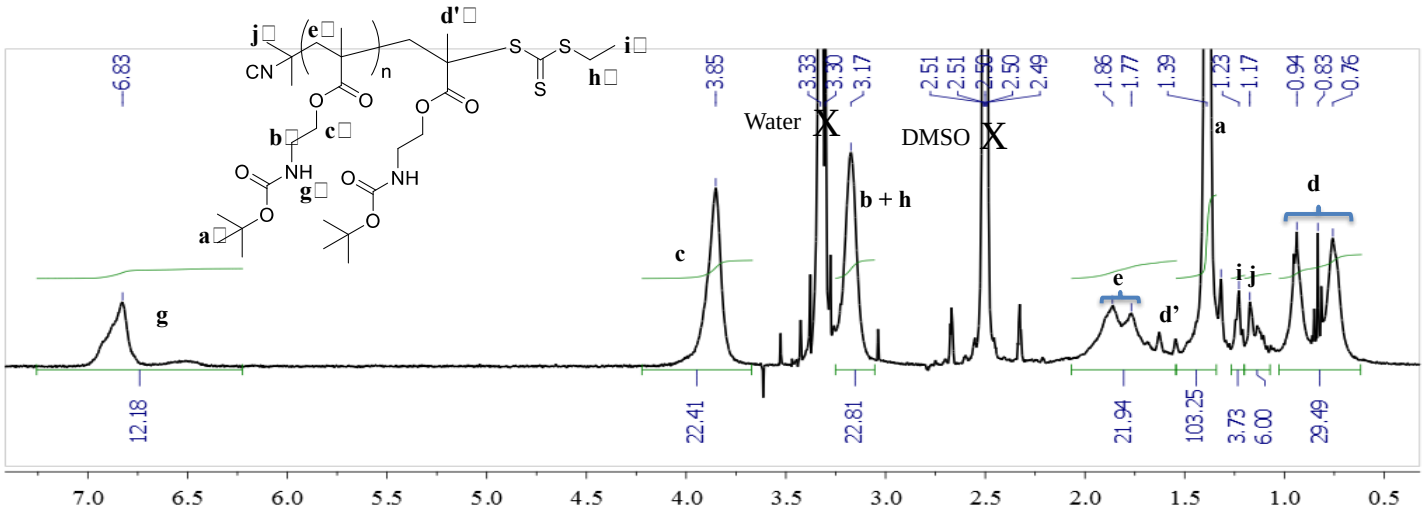


Figure S1. ^{1}H NMR spectra of monomer (top) and RAFT agent (CPETC) (bottom)

Boc – $P_{9.9}$ (DMSO - d6)



Boc – P_{11} (DMSO – d6)



Boc – P_{19} (DMSO – d6)

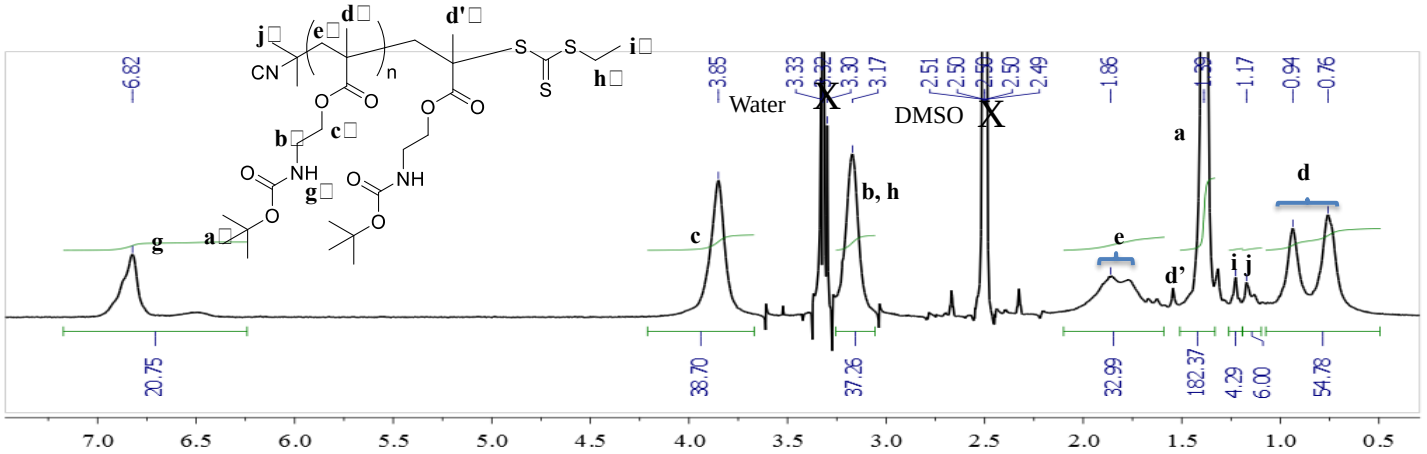
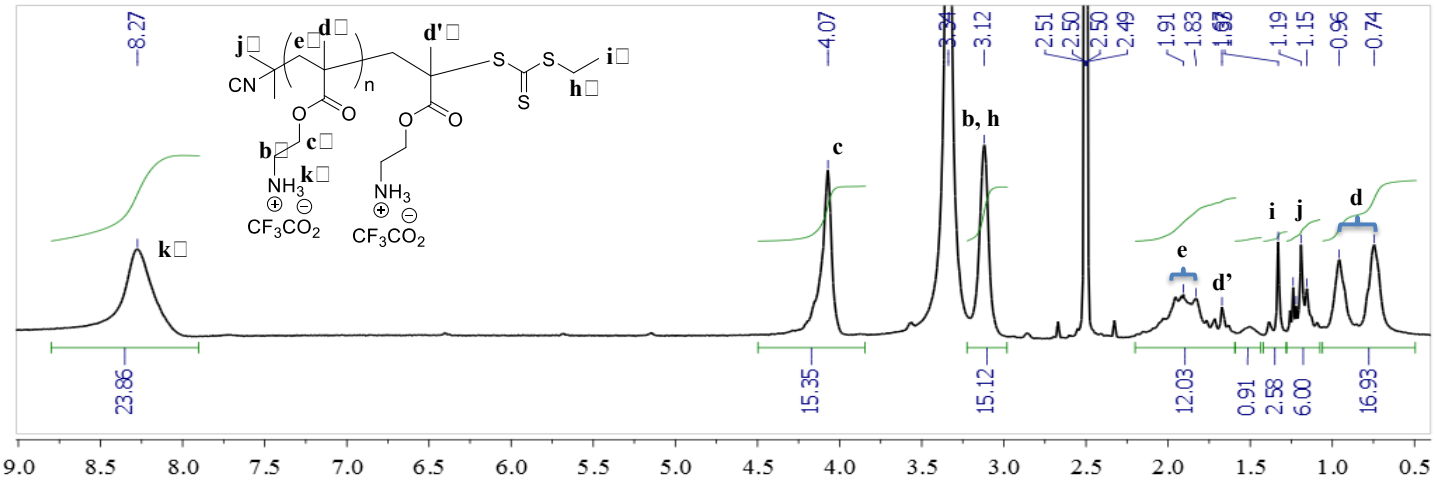
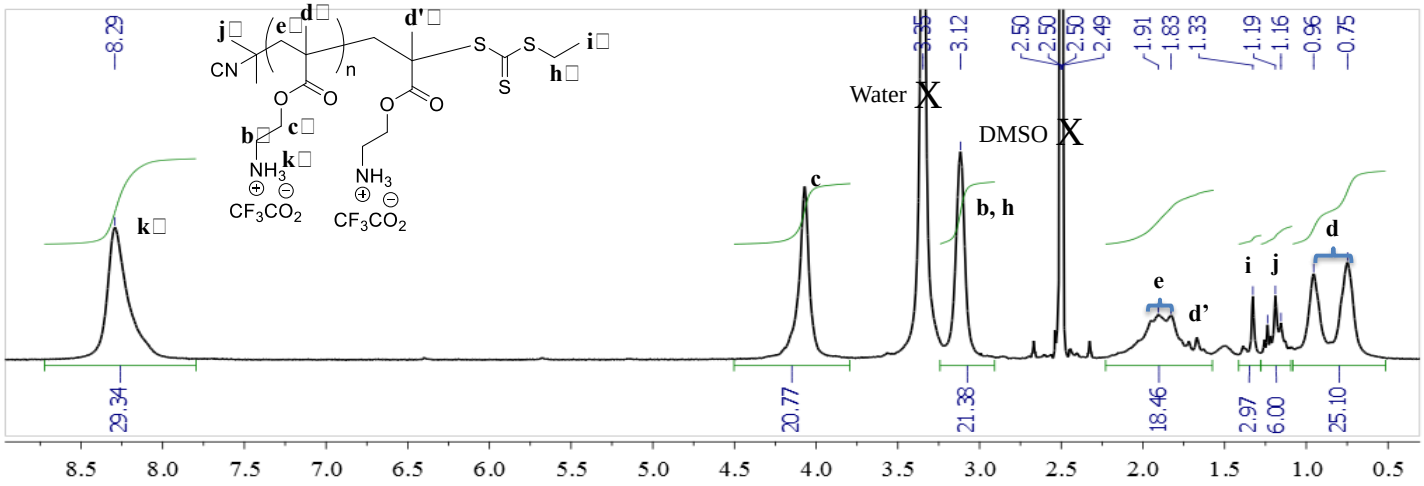


Figure S2. ^{1}H NMR spectra of Boc-protected polymers

$P_{7.7}$ – Deprotected (DMSO-d6)



P_{10} – Deprotected (DMSO – d6)



P_{12} – Deprotected (DMSO – d6)

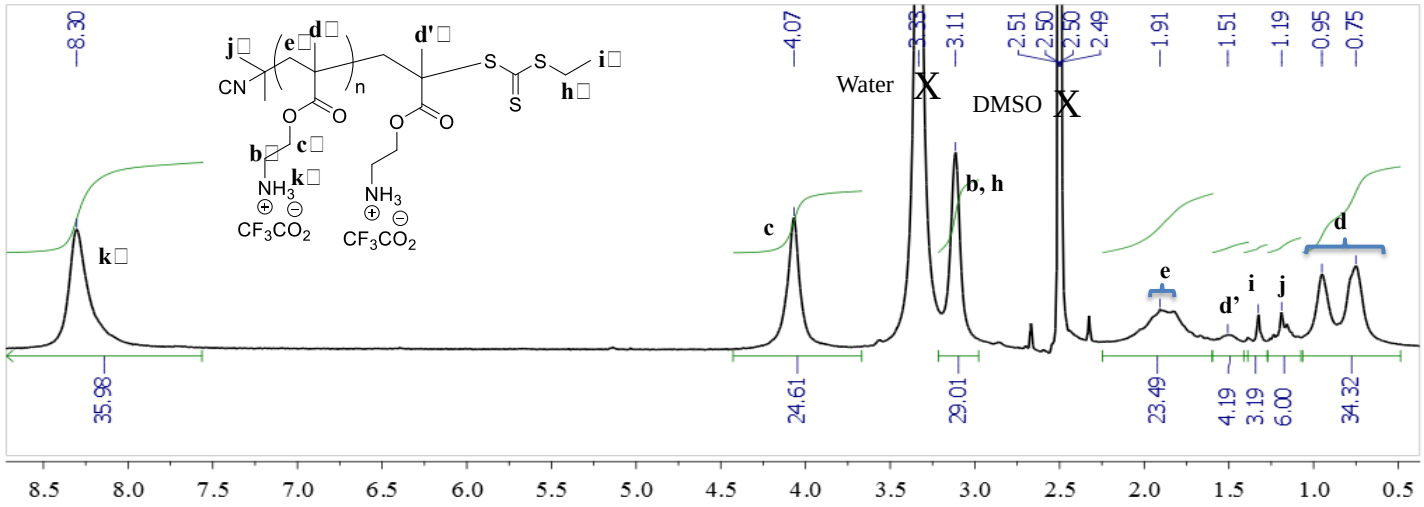


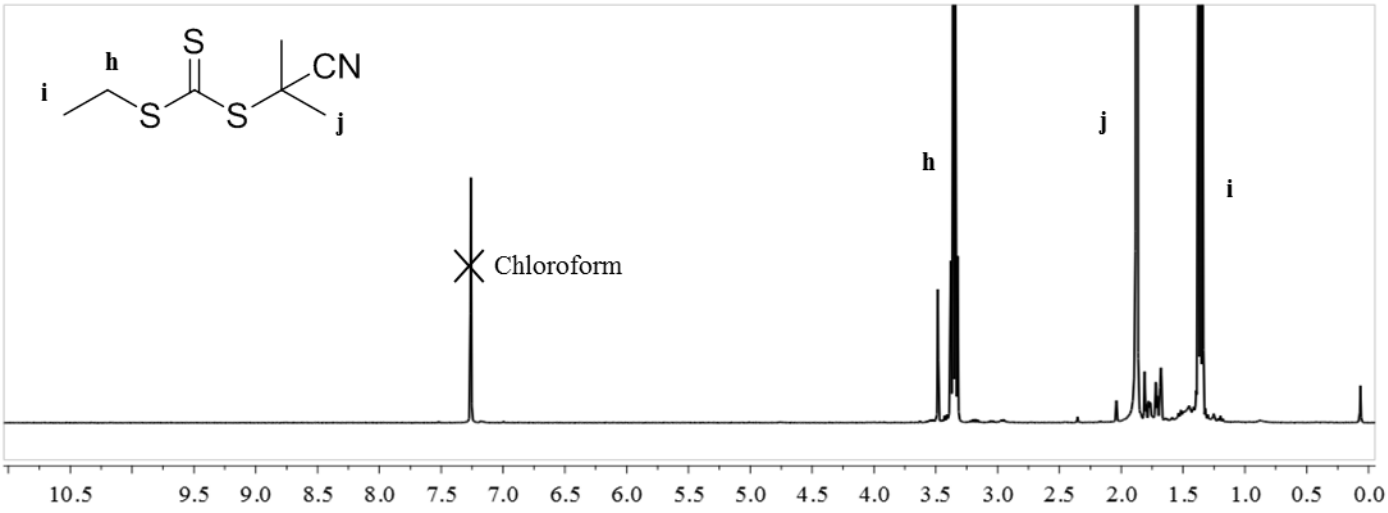
Figure S3. ^{1}H NMR spectra of deprotected cationic homopolymers after treatment in TFA.

2. CPETC after exposure to TFA

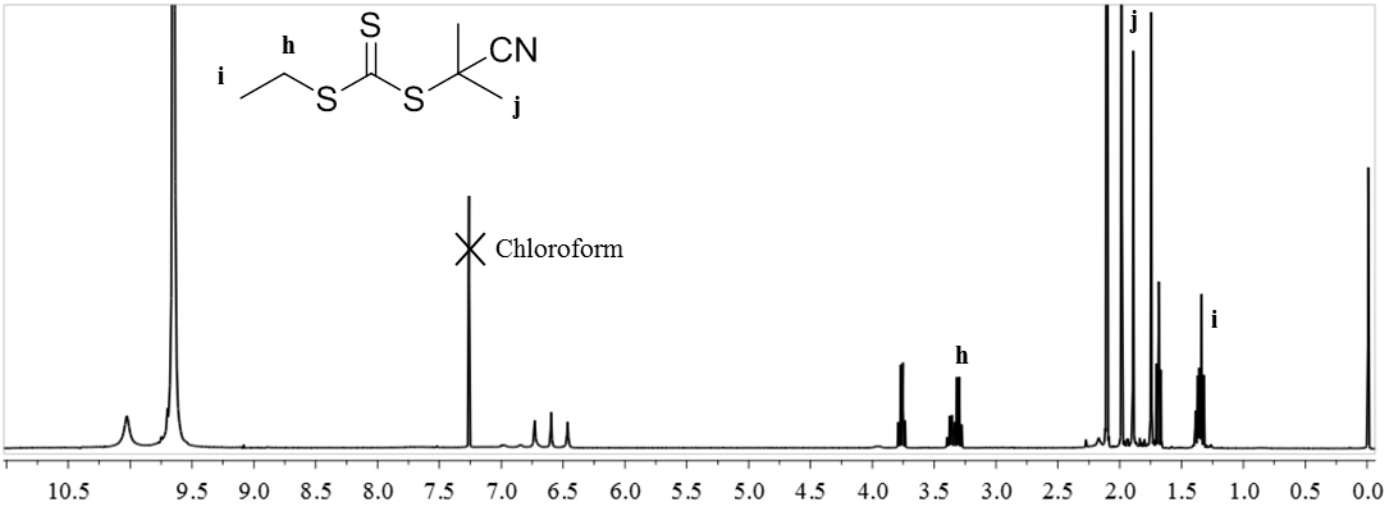
Trifluoroacetic acid (TFA) (100 μL) was added to 2-cyanoprop-2-yl ethyl trithiocarbonate (CPETC) (20.5 mg, 0.1 mmol). The reaction mixture was stirred at room temperature. Aliquots were removed at 0, 5 and 30 minutes, and the reaction was monitored by ^{1}H NMR analysis in chloroform.

The peaks of CPETC (3.35, 1.88, and 1.35 ppm) were monitored upon the addition of TFA (Fig. S4). After 5 minutes, additional peaks appeared, indicating the formation of products likely due to degradation of CPETC. After 30 minutes, the multiplets around 3.3 and 1.34 ppm remain, while the singlet at 1.88 ppm has disappeared. This demonstrates that the trithioester end groups of polymers might decompose during the deprotection of boc groups in the side chains of polymers under the acidic condition, possibly as a result of hydrolysis although the detailed mechanism of reaction is not clear at this point.

CPETC (t = 0)



CPETC + TFA (t = 5 min)



CPETC + TFA (t = 30 min)

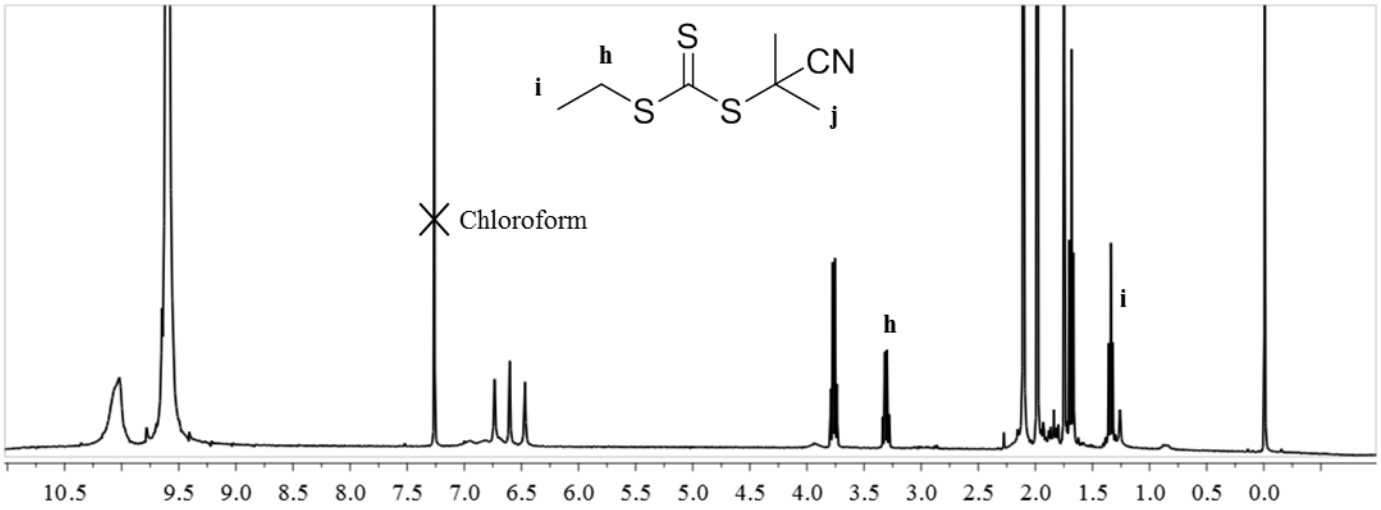


Figure S4. ^{1}H NMR spectra of CPETC after exposure to TFA

3. Membrane Depolarization Assay

Cytoplasmic membrane disruption was evaluated for polymers against *S. aureus* using the membrane potential sensitive dye $DISC_3(5)$. A single colony of *S. aureus* was inoculated in MHB for 18h at 37°C, and mid-logarithmic phase cells (OD_{600} = 0.5 – 0.6) were collected. Cells were then resuspended in buffer (5 mM HEPES, 5 mM sucrose, 100 mM KCl, pH 7.2) to OD_{600} = 0.05. A stock solution of $DISC_3(5)$ in ethanol was added to *S. aureus* suspension (3 mL). The final dye concentration is 0.5 μM. The cell suspension with $DISC_3(5)$ (0.5 μM) was stirred at room temperature until the stable reduction in fluorescence intensity was achieved due to quenching upon accumulation of dye on the *S. aureus* membrane. At 500 s, a solution of polymer (various concentrations, 5 μL) in HEPES buffer was added to the bacterial suspension to give the final concentration (0.5 – 8x MIC). The fluorescence intensity was monitored with excitation and emission wavelengths of 622 and 670 nm, respectively. As a control, the changes in fluorescence intensity of dye and polymers mixtures in buffer were determined.

As a control, the changes in fluorescence intensity of $DiSC_3(5)$ and polymer mixtures in the absence of bacteria were determined (Figure S5).

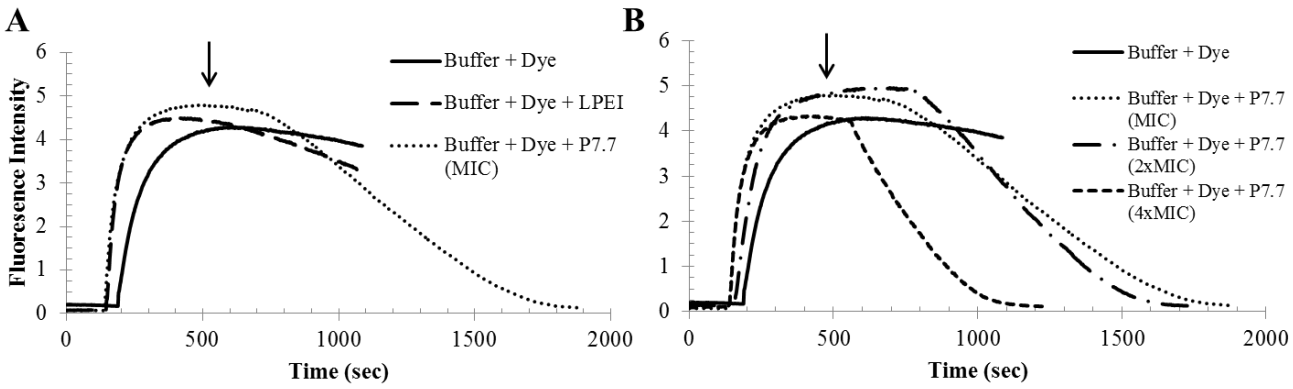


Figure S5. Fluorescence intensity of $DiSC_3(5)$ in the presence of AEMP and LPEI. The fluorescence intensity was determined by monitoring the absorbance of dye mixed with polymers in the absence of *S. aureus*. Dye was added into PBS buffer (final concentration 0.5 μM) and mixed with polymers at their MIC concentrations. A) Shows the dye alone, and mixed separately with LPEI and $P_{7.7}$ at their respective MICs. B) Shows the dye mixed with $P_{7.7}$ at various concentrations. Open arrows indicate the point of polymer addition.

Figure S5 shows the change in absorbance of the dye mixed with polymers in the absence of *S. aureus*. Both LPEI and $P_{7.7}$ caused a decrease in fluorescence at a much faster rate than the dye self-quenches in the

absence of bacteria (Fig. S5A). As the concentration of $P_{7.7}$ increased, the rate of fluorescence quenching increased (Fig. S5B). It is likely that the polymer binds to the dye, and the interaction between the polymer and dye causes quenching of fluorescence from the dye although the molecular mechanism is not clear at this point.

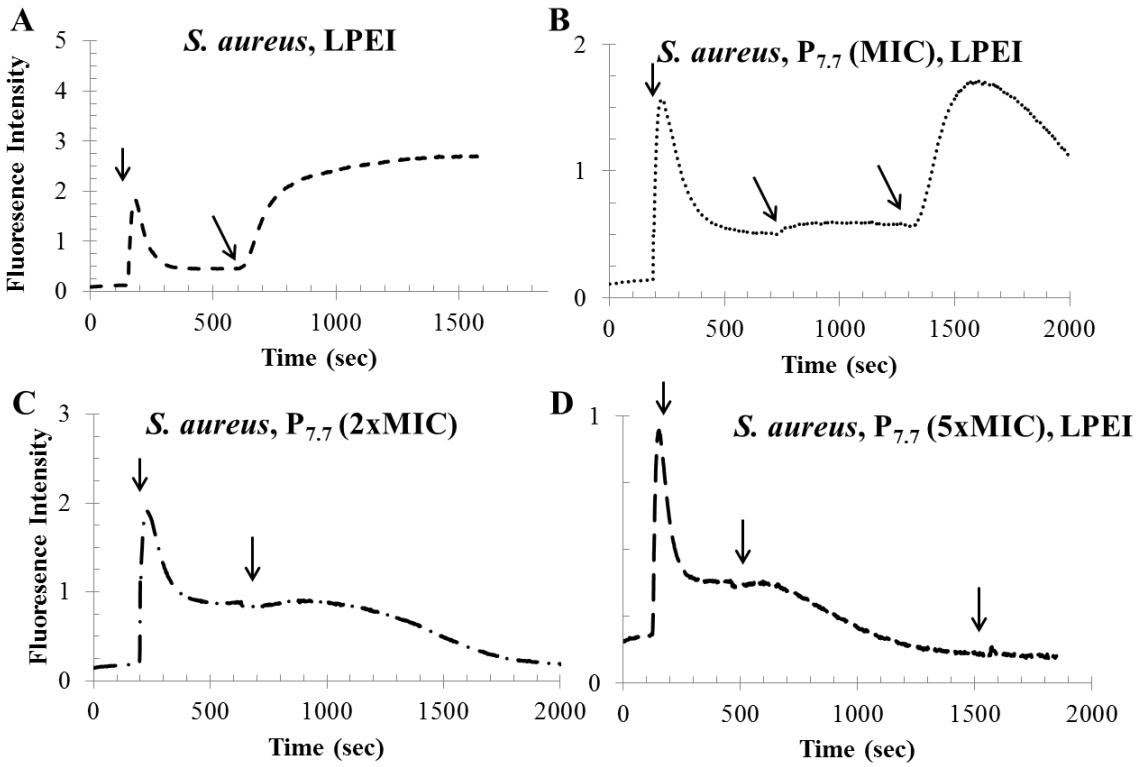


Figure S6. *S. aureus* membrane depolarization by LPEI and AEMP. First arrow indicates dye introduction, 2nd arrow indicates polymer addition. For B and D, third arrow indicates the addition of LPEI.

Figure S6 shows the change in fluorescence induced by the polymer mixtures in the presence of *S. aureus*. Figure S6A demonstrates the change in fluorescence in the presence of LPEI, with results similar to what we have previously demonstrated.[1] LPEI caused membrane depolarization, as demonstrated by the increase in fluorescence from $DISC_3(5)$ after its addition to the *S. aureus* solution. Figure S6 B-D demonstrates the change in fluorescence from $DISC_3(5)$ bound to *S. aureus* upon the addition of various concentrations of $P_{7.7}$. Only a slight change in fluorescence was observed at polymer concentrations 1x, 2x or 5x the MIC of $P_{7.7}$. However, as the polymer reduced the fluorescence of $DISC_3(5)$ in solution (Fig. S5), the results could be due to the fluorescence quenching by the polymer rather than the inability of polymer to cause membrane depolarization. Therefore, this approach cannot determine if this lack of substantial change in fluorescence upon introduction of

$P_{7.7}$ shows that $P_{7.7}$ doesn't cause membrane depolarization or if it is a result of fluorescence quenching by the polymer. In addition, a third arrow marks the addition of LPEI, a known membrane depolarizer, for $P_{7.7}$ at the MIC and 5x the MIC (Fig. S6B & D). After the addition of LPEI for $P_{7.7}$ at the MIC, the fluorescence increased, indicating that membrane depolarization did occur (Fig. S6B). However after the addition of LPEI to $P_{7.7}$ at 5x the MIC, there is no change in fluorescence (Fig. S6D). This is likely either because the high concentration of $P_{7.7}$ quenches fluorescence caused by the LPEI or $P_{7.7}$ in the cell wall prevents membrane depolarization possibly by inhibiting the diffusion of LPEI through the cell wall to the cell membrane. It cannot be ruled out that $P_{7.7}$ binds to LPEI, which sequesters the LPEI from solution.

In conclusion, the membrane depolarization assay using $DiSC_3(5)$ does not provide quantitative results to determine the ability of $P_{7.7}$ to cause membrane depolarization in S. aureus due to the strong quenching of fluorescence from $DiSC_3(5)$ by the polymer.

4. Cell Leakage Assay

Leakage of UV-absorbing cellular components from *S. aureus* and *E. coli* upon treatment with $P_{7.7}$ was measured as a measure of membrane disruption by the polymer. The bacteria were regrown in MHB to give an $OD_{600} > 0.8$. The bacterial cells were harvested, and the pellets were washed with PBS. The cells were then re-suspended in PBS buffer. Cells were then incubated with the compound of interest ($P_{7.7}$, lysostaphin, melittin, CTAB, or PBS for control) with shaking at 37°C. 2 mL of solution was removed at 2 hours, and centrifuged at 14,000 rpm for 10 minutes to afford cell-free supernatant. The absorbance of cell-free supernatant was measured at 260 and 600 nm.

Table S1. Absorbance of assay solutions

	E. coli	*S. aureus*
Starting OD_{600}	0.250	0.250
OD_{600} after centrifugation		
PBS	0.0299	0.0288
PBS, bacteria	0.0321	0.0255
PBS, bacteria, CTAB	0.0278	0.0276
PBS, bacteria, Lysostaphin	0.0271	0.0264
PBS, bacteria, Melittin	0.0309	0.0289
PBS, bacteria, $P_{7.7}$	0.0281	0.0277

CTAB, lysostaphin, melittin, and $P_{7.7}$ were tested. PBS buffer without any compound was used as a control. All test compounds were used at a final concentration of at least 3x their MIC in the respective bacteria. After 2 hours, the assay solution was centrifuged at 14,000 rpm for 10 minutes. The OD_{600} values of assay solutions are close to PBS, indicating the supernatant was cell-free after centrifugation. The absorbance of the supernatant was measured at 260 nm to detect UV-absorbing cellular components. As a background, the absorbance of test compounds in PBS without bacteria was determined, and subtracted from the absorbance reading in the presence of bacteria at 260 nm. The corrected absorbance for *E. coli* and *S. aureus* are shown in Figure S7.

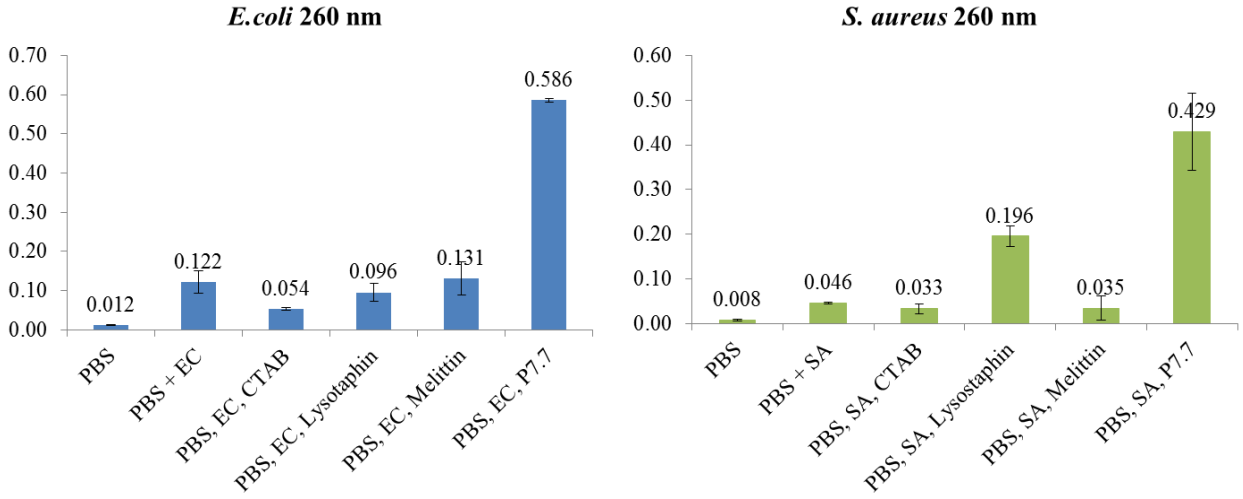


Figure S7. UV-absorbing cellular components from *S. aureus* and *E. coli* upon treatment with $P_{7.7}$ and other controls after 2 hours.

PBS was used as a negative control, as it should cause no leakage of cellular components from the bacterial membrane. CTAB (a cationic surfactant) and melittin (lytic peptide) were used for comparison. Lysostaphin (an endopeptidase capable of cleaving the crosslinking pentaglycin bridges of *Staphylococci* for cell lysis) were used as a positive control for 100% leakage for *S. aureus*. $P_{7.7}$ showed a large increase in absorbance at 260 nm for both bacteria after 2 hours of treatment. It appears that $P_{7.7}$ causes strong membrane permeabilization. However, the absorbance is significantly higher than the absorbance for lysopstaphin and melittin controls that we expected to give 100% lysis of bacterial cells and subsequent protein leakage. Therefore, because of the amphiphilic nature of the polymer, $P_{7.7}$ is likely to bind to cellular components such as proteins and lipids, which may cause formation of small aggregates. The small aggregates may result in apparent increase in the UV absorbance due to light scattering. The polymer aggregates may also change the environment of UV-sensitive functional groups of proteins, increasing the absorbance.

In conclusion, the membrane permeabilization assay monitoring UV-absorbing cellular components does not provide quantitative results to determine the ability of $P_{7.7}$ to cause membrane permeabilization in bacteria due to the increased absorbance, which is likely to reflect the aggregation of cellular components with polymer.

5. ***In vivo* testing**

As a preliminary assessment of the *in vivo* topical treatment of *S. aureus* infections using AEMPs, we chose a cotton rat nasal *S. aureus* colonization model. Two trials were performed: trial 1 involved 5 animals for $P_{7.7}$, 3 animals for mupirocin, and 3 animals for PBS buffer (control); trial 2 involved 5 animals for P_{10}, 5 animals for mupirocin, and 5 animals for PBS control. The data from all trials are given below.

Table S2. In vivo testing data

	Number of *S. aureus* BB2146 cells isolated			
	PBS Control	**Mupirocin**	**$P_{7.7}$**	**P_{10}**
Trial 1	3169	50	0	-
	2901	179	0	-
	3595	102	6	-
			0	-
			19	-
Trial 2	2019	3120	-	0
	2593	77	-	0
	3740	983	-	11
	1902	587	-	0
	2983	1276	-	65

1. Gibney, K. A.; Sovadinova, I.; Lopez, A. I.; Urban, M.; Ridgway, Z.; Caputo, G. A.; Kuroda, K., Poly(ethylene imine)s as Antimicrobial Agents with Selective Activity. *Macromol. Biosci.* **2012,** *12* (9), 1279-1289.