Electronic Supplementary Information

Rapid bactericidal activity of amphiphilic polyacrylate terpolymer system comprised of same centered comonomers with 2-carbon and 6-carbon spacer arms and an uncharged repeating unit

Kamia Punia, Ashish Punia, Kaushiki Chatterjee, Sumit Mukherjee, Jimmie Fata, Probal Banerjee, Krishnaswami Raja and Nan-Loh Yang

Ph.D. Program in Chemistry at the Graduate Center of the City University of New York, New York, NY 10016, United States
Ph.D. Program in Biochemistry at the Graduate Center of the City University of New York, New York, NY 10016, United States
Department of Biology, College of Staten Island, the City University of New York, Staten Island, NY 10314, United States

S 1. $^1$H NMR spectra of monomers and copolymers

$6$-((tert-butoxycarbonyl)amino)hexyl acrylate (Monomer M6) $^1$H NMR (600 MHz, CDCl$_3$): $\delta$ 1.28-1.36 (m, 4H), 1.37-1.42 (s, 9H), 1.42-1.48 (m, 2H), 1.63 (m, 2H), 3.06 (s, 2H), 4.10 (t, 2H), 5.77 (dd, 1H), 6.07 (q, 1H), 6.35 (dd, 1H).

$2$-((tert-butoxycarbonyl)methylamino)ethyl acrylate (Monomer M2) $^1$H NMR (600 MHz, CDCl$_3$): $\delta$ 1.41 (s, 9H), 2.88 (s, 3H), 3.48 (s, 2H), 4.23 (s, 2H), 5.81 (d, 1H), 6.09 (q, 1H), 6.38 (d, 1H).

PolyM6-M2 $^1$H NMR (600 MHz, D$_2$O): $\delta$ 1.36-1.47 (bs, 75H), 1.56-2.6 (bm, 162H), 2.72 (s, 2H), 2.80-2.86 (bs, 33H), 2.99-3.04 (bs, 36H), 3.37-3.45 (bs, 22H), 3.74 (s, 3H), 4.00-4.21 (bs, 36H), and 4.31-4.84 (bs, 22H).

PolyM-4% $^1$H NMR (600 MHz, D$_2$O): $\delta$ 1.29-1.39 (bs, 60H), 1.49-2.54 (bm, 135H), 2.64 (s, 2H), 2.71-2.78 (bs, 25H), 2.91-2.97 (bs, 28H), 3.30-3.38 (bs, 16H), 3.61-3.64 (bs, 2H), 3.67 (s, 3H), 3.96-4.12 (bs, 28H), and 4.23-4.37 (bs, 16H).
PolyM-12% $^1^H$ NMR (600 MHz, D$_2$O): $\delta$ 1.29-1.39 (bs, 65H), 1.49-2.54 (bm, 142H), 2.64 (s, 2H), 2.72-2.79 (bs, 21H), 2.91-2.97 (bs, 31H), 3.30-3.38 (bs, 14H), 3.61-3.66 (bs, 7H), 3.67 (s, 3H), 3.94-4.12 (bs, 31H), and 4.23-4.37 (bs, 14H).

PolyM-24% $^1^H$ NMR (600 MHz, D$_2$O): $\delta$ 1.29-1.42 (bs, 65H), 1.49-2.54 (bm, 143H), 2.65 (s, 2H), 2.72-2.79 (bs, 12H), 2.91-2.97 (bs, 30H), 3.30-3.38 (bs, 7H), 3.61-3.66 (bs, 15H), 3.67 (s, 3H), 3.94-4.12 (bs, 30H), and 4.23-4.37 (bs, 7H).

PolyE-4% $^1^H$ NMR (600 MHz, D$_2$O): $\delta$ 1.23-1.32 (bs, 3H) $\delta$ 1.36-1.44 (bs, 66H), 1.58-2.58 (bm, 145H), 2.71 (s, 2H), 2.79-2.83 (bs, 26H), 2.96-3.02 (bs, 33H), 3.36-3.44 (bs, 17H), 3.72 (s, 3H), 4.01-4.19 (bs, 33H), and 4.30-4.43 (bs, 17H).

PolyE-24% $^1^H$ NMR (600 MHz, D$_2$O): $\delta$ 1.21-1.32 (bs, 20H) $\delta$ 1.34-1.46 (bs, 92H), 1.51-2.51 (bm, 198H), 2.70 (s, 2H), 2.77-2.81 (bs, 16H), 2.97-3.04 (bs, 40H), 3.36-3.42 (bs, 10H), 3.72 (s, 3H), 3.98-4.21 (bs, 57), and 4.29-4.43 (bs, 10H).

PolyB-4% $^1^H$ NMR (600 MHz, D$_2$O): $\delta$ 0.93-1.00 (bs, 3H) $\delta$ 1.36-1.47 (bs, 61H), 1.56-2.60 (bm, 136H), 2.79 (s, 2H), 2.79-2.87 (bs, 27H), 2.98-3.05 (bs, 30H), 3.36-3.48 (bs, 18H), 3.74 (s, 3H), 4.01-4.21 (bs, 31H), and 4.31-4.47 (bs, 18H).

PolyB-12% $^1^H$ NMR (600 MHz, D$_2$O): $\delta$ 0.93-1.00 (bs, 10H) $\delta$ 1.34-1.47 (bs, 83H), 1.52-2.60 (bm, 171H), 2.79 (s, 2H), 2.79-2.84 (bs, 24H), 2.96-3.03 (bs, 37H), 3.34-3.44 (bs, 14H), 3.72 (s, 3H), 4.01-4.19 (bs, 44H), and 4.29-4.44 (bs, 14H).

PolyB-24% $^1^H$ NMR (600 MHz, D$_2$O): $\delta$ 0.91-1.03 (bs, 22H) $\delta$ 1.34-1.47 (bs, 92H), 1.49-2.57 (bm, 181H), 2.70 (s, 2H), 2.78-2.84 (bs, 15H), 2.96-3.03 (bs, 37H), 3.33-3.45 (bs, 9H), 3.72 (s, 3H), 3.96-4.21 (bs, 52H), and 4.29-4.43 (bs, 9H).
S 2. Determination of actual mole percentage of the repeating units in polymer

S 2a. PolyM6-M2: In Figure S 2a, a broad single peak at δ 2.99-3.04 (a) belongs to methylene protons in M6 monomer repeating unit. Methylene protons in M2 monomer repeating unit (k) give a chemical shift at δ 3.37-3.45. Mole percentages of the repeating units are calculated as below assuming chain transfer as mode of termination:

Mol% of M6 repeat unit: $(36/2) \div (18 + 11) = 62\%$

Mol% of M2 repeat unit: $(22/2) \div (18 + 11) = 38\%$

Figure S 2a. $^1$H NMR spectrum of PolyM6-M2
S 2b. PolyM-4%: In Figure S 2b, a broad single peak at δ 2.91-2.97 (a) belongs to methylene protons in M6 monomer repeating unit. Methylene protons in M2 monomer repeating unit (k) give a chemical shift at δ 3.30-3.38. A broad single peak at δ 3.61-3.64 corresponds to methoxy protons (p) in methyl acrylate monomer. Mole percentages of the repeating units are calculated as below assuming chain transfer as mode of termination:

Mol% of M6 repeat unit: \( \frac{28/2}{14 + 8 + 0.67} = 62\% \)

Mol% of M2 repeat unit: \( \frac{16/2}{14 + 8 + 0.67} = 35\% \)

Mol% of methyl acrylate repeat unit: \( \frac{2/3}{14 + 8 + 0.67} = 3\% \)

Figure S 2b. \(^1\)H NMR spectrum of PolyM-4%
S 2c. PolyM-12%: In Figure S 2c, a broad single peak at δ 2.91-2.97 (a) belongs to methylene protons in M6 monomer repeating unit. Methylene protons in M2 monomer repeating unit (k) give a chemical shift at δ 3.30-3.38. A broad single peak at δ 3.61-3.66 corresponds to methoxy protons (p) in methyl acrylate monomer. Mole percentages of the repeating units are calculated as below assuming chain transfer as mode of termination:

Mol% of M6 repeat unit: \( (31/2) \div (15.5 + 7 + 2.33) = 62\% \)

Mol% of M2 repeat unit: \( (14/2) \div (15.5 + 7 + 2.33) = 28\% \)

Mol% of methyl acrylate repeat unit: \( 7/3 \div (15.5 + 7 + 2.33) = 10\% \)

Figure S 2c. \(^1\)H NMR spectrum of PolyM-12%
**S 2d. PolyM-24%**: In Figure S 2d, a broad single peak at δ 2.91-2.97 (a) belongs to methylene protons in M6 monomer repeating unit. Methylene protons in M2 monomer repeating unit (k) give a chemical shift at δ 3.30-3.38. A broad single peak at δ 3.61-3.66 corresponds to methoxy protons (p) in methyl acrylate monomer. Mole percentages of the repeating units are calculated as below assuming chain transfer as mode of termination:

Mol% of M6 repeat unit: \( \frac{30/2}{15 + 3.5 + 5} = 64\% \)

Mol% of M2 repeat unit: \( \frac{7/2}{15 + 3.5 + 5} = 15\% \)

Mol% of methyl acrylate repeat unit: \( \frac{15/3}{15 + 3.5 + 5} = 21\% \)

![PolyM-24%](image)

Figure S 2d. \(^1\)H NMR spectrum of PolyM-24%
**S 2e. PolyE-4%**: In Figure S 2e, a broad single peak at δ 2.96-3.02 (a) belongs to methylene protons in M6 monomer repeating unit. Methylene protons in M2 monomer repeating unit (k) give a chemical shift at δ 3.36-3.44. A broad single peak at δ 1.23-1.32 corresponds to methyl protons (p) in ethyl acrylate monomer. Mole percentages of the repeating units are calculated as below assuming chain transfer as mode of termination:

Mol% of M6 repeat unit: \( \frac{33/2}{16.5 + 8.5 + 1} = 63\% \)

Mol% of M2 repeat unit: \( \frac{17/2}{16.5 + 8.5 + 1} = 33\% \)

Mol% of ethyl acrylate repeat unit: \( \frac{3/3}{16.5 + 8.5 + 1} = 4\% \)

![PolyE-4% structure](image)

Figure S 2e. \(^1\)H NMR spectrum of PolyE-4%
S 2f. PolyE-12%: In Figure S 2f, a broad single peak at δ 2.97-3.03 (a) belongs to methylene protons in M6 monomer repeating unit. Methylene protons in M2 monomer repeating unit (k) give a chemical shift at δ 3.36-3.44. A broad single peak at δ 1.23-1.32 corresponds to methyl protons (p) in ethyl acrylate monomer. Mole percentages of the repeating units are calculated as below assuming chain transfer as mode of termination:

Mol% of M6 repeat unit: \( \frac{46/2}{23 + 9.5 + 4.33} = 62\% \)
Mol% of M2 repeat unit: \( \frac{19/2}{16.5 + 8.5 + 1} = 26\% \)
Mol% of ethyl acrylate repeat unit: \( \frac{13/3}{16.5 + 8.5 + 1} = 12\% \)

Figure S 2f. \(^1\)H NMR spectrum of PolyE-12%
S 2g. PolyE-12%: In Figure S 2g, a broad single peak at $\delta$ 2.97-3.04 (a) belongs to methylene protons in M6 monomer repeating unit. Methylene protons in M2 monomer repeating unit (k) give a chemical shift at $\delta$ 3.36-3.42. A broad single peak at $\delta$ 1.21-1.32 corresponds to methyl protons (p) in ethyl acrylate monomer. Mole percentages of the repeating units are calculated as below assuming chain transfer as mode of termination:

- Mol% of M6 repeat unit: \( \frac{40}{2} \div (20 + 5 + 6.7) = 63\% \)
- Mol% of M2 repeat unit: \( \frac{10}{2} \div (20 + 5 + 6.7) = 16\% \)
- Mol% of ethyl acrylate repeat unit: \( \frac{20}{3} \div (20 + 5 + 6.7) = 21\% \)

Figure S 2g. $^1$H NMR spectrum of PolyE-24%
**S 2h. PolyB-4%:** In Figure S 2h, a broad single peak at δ 2.98-3.05 (a) belongs to methylene protons in M6 monomer repeating unit. Methylene protons in M2 monomer repeating unit (k) give a chemical shift at δ 3.36-3.48. A broad single peak at δ 0.93-1.00 corresponds to methyl protons (p) in butyl acrylate monomer. Mole percentages of the repeating units are calculated as below assuming chain transfer as mode of termination:

Mol% of M6 repeat unit: \((30/2) ÷ (15 + 9 +1) = 60\%\)
Mol% of M2 repeat unit: \((18/2) ÷ (15 + 9 +1) = 36\%\)
Mol% of butyl acrylate repeat unit: \(3/3 ÷ (15 + 9 +1) = 4\%\)

![PolyB-4%](image)

Figure S 2h. \(^1\)H NMR spectrum of PolyB-4%
S 2i. PolyB-12%: In Figure S 2i, a broad single peak at δ 2.96-3.03 (a) belongs to methylene protons in M6 monomer repeating unit. Methylene protons in M2 monomer repeating unit (k) give a chemical shift at δ 3.34-3.44. A broad single peak at δ 0.93-1.00 corresponds to methyl protons (p) in butyl acrylate monomer. Mole percentages of the repeating units are calculated as below assuming chain transfer as mode of termination:

Mol% of M6 repeat unit: \( \frac{37/2}{18.5 + 7 + 3.33} = 64\% \)

Mol% of M2 repeat unit: \( \frac{14/2}{18 + 7.5 + 3.33} = 25\% \)

Mol% of butyl acrylate repeat unit: \( \frac{10/3}{18 + 7.5 + 3.33} = 11\% \)

Figure S 2i. \(^1\)H NMR spectrum of PolyB-12%
S 2j. PolyB-24%: In Figure S 2j, a broad single peak at δ 2.96-3.03 (a) belongs to methylene protons in M6 monomer repeating unit. Methylene protons in M2 monomer repeating unit (k) give a chemical shift at δ 3.33-3.45. A broad single peak at δ 0.91-1.03 corresponds to methyl protons (p) in butyl acrylate monomer. Mole percentages of the repeating units are calculated as below assuming chain transfer as mode of termination:

Mol% of M6 repeat unit: \( \frac{37/2}{18.5 + 4.5 + 7.33} = 61\% \)

Mol% of M2 repeat unit: \( \frac{9/2}{18.5 + 4.5 + 7} = 15\% \)

Mol% of butyl acrylate repeat unit: \( \frac{22/3}{18.5 + 4.5 + 7} = 24\% \)

Figure S 2j. \(^1\)H NMR spectrum of PolyB-24%
S 3. Estimation of molecular weight through gel permeation chromatography (GPC)

Figure S 3.1. Gel permeation chromatography elution curves of copolymers

Figure S 3.2. Polystyrene standard calibration curve used for the estimation of molecular weights of copolymer samples by GPC
S 4. Time-dependent bacterial killing efficiency of PolyE-12% copolymer

Figure S 4. Time-dependent killing efficiency analysis of PolyE-12% against a) *E. coli* and b) *S. aureus*. Results shown here are the averages of two experiments (size of error bars is negligible).