Supporting Information

Beyond Amphiphilic Balance: Changing Subunit Stereochemistry Alters the Pore-Forming Activity of Nylon-3 Polymers

Lei Liu1†, Kevin C. Courtney2,4†, Sean W. Huth1, Leslie A. Rank1, Bernard Weisblum3, Edwin R. Chapman2,4*, Samuel H. Gellman1*

1Department of Chemistry, University of Wisconsin – Madison
2Department of Neuroscience, University of Wisconsin – Madison
3Department of Pharmacology, University of Wisconsin – Madison
4Howard Hughes Medical Institute

† These authors contributed equally to this work.
* To whom correspondence should be addressed.

Table of Contents

Materials and Instrumentation ................................................................. S2
Monomer Synthesis Procedures ................................................................. S3
UPLC Characterization ............................................................................. S19
Polymer Synthesis Procedures ................................................................. S21
NMR Spectral and GPC Data for Polymers ................................................. S23
Assays for Nylon-3 Polymers ................................................................... S39
Materials and instrumentation

Chlorosulfonyl isocyanate was purchased from TCI America. DOPC and DOPS lipids were purchased from Avanti Polar Lipids. All other chemicals were purchased from Sigma-Aldrich and used without purification. For liposome experiments, the ultra-low attachment polystyrene 96-well plates and ITO-coated glass slides were purchased from Sigma Aldrich. Melittin was synthesized on an automated microwave peptide synthesizer – Liberty Blue, manufactured by CEM. Proton ($^1\text{H}$) and Carbon ($^{13}\text{C}$) Nuclear Magnetic Resonance (NMR) spectra were obtained on a Bruker Avance III spectrometer at 400 and 101 MHz, respectively, or on a Bruker Avance III spectrometer at 500 and 126 MHz, respectively. All $^1\text{H}$ chemical shifts are reported in ppm using trimethylsilane (TMS) or the solvent as the internal standards (TMS at 0 ppm, or $\text{D}_2\text{O}$ at 4.790 ppm). All $^{13}\text{C}$ chemical shifts are reported in ppm using solvent as the internal standard (CDCl$_3$ at 77.160 ppm). All ultraperformance liquid chromatography (UPLC) chromatograms were obtained with a Waters Acquity UPLC using a BEH C18 column, with a 1.7 µm particle size and dimensions 2.1 × 50 mm. The UPLC chromatograms were based on UV detections at 210 nm. Polymerization reactions were carried out in an MBraun LABstar glove box under a nitrogen atmosphere at ambient temperature. N,N-Dimethylacetamide (DMAc) gel-permeation chromatography (GPC) was performed using two Waters styragel HR 4E columns (particle size 5 µm) linked in series on a Waters LC instrument equipped with a refractive index detector (Waters 2410). DMAc containing 10 mM LiBr was used as the mobile phase at a flow rate of 1 mL/min at 80°C. Number-average molecular weight (Mn) and dispersity (Đ) were calculated using Empower software and calibration curves from polymethacrylate standards in DMAc (10 mM LiBr at 80°C). EasiVial polymethyl methacrylate (PMMA) standards for GPC column calibration (PL2020-0200) were obtained from Polymer Varian (Palo Alto, CA). Tetrahydrofuran (THF) GPC analysis involved two Waters columns (Styragel HR 4E and HR 2 particle size 5 µm) linked in series. The Waters liquid chromatography unit ( Alliance) was equipped with a multangle light scattering detector (Wyatt miniDAWN TREOS, 658 nm) and a refractive index detector (Wyatt Optilab-rEX, 658 nm). Mn and Đ were calculated using ASTRA software using a dn/dc value of 0.1 mL/g. Mass spectra were acquired using a Thermo ScientificTM Q ExactiveTM hybrid quadrupole-Orbitrap mass spectrometer (electrospray ionization-quadrupole-ion trap (orbitrap)-mass spectrometer, ESI-Q-IT-MS).
Monomer Synthesis Procedures

**ME-trans β Synthesis**

![Scheme S1](image)

**Scheme S1**: Synthesis of the β-lactam (compound 5) that generates the ME-trans subunit in nylon-3 polymers.

**(E)-1-Bromo-2-pentene (2)**: To an oven-dried round-bottom flask that was purged with N\textsubscript{2} gas was added anhydrous diethyl ether (200 mL). Compound 1 (10.0 g, 116 mmol, 1.0 equiv.) was dissolved in diethyl ether under N\textsubscript{2} atmosphere. Then phosphorus tribromide (13.1 mL, 139 mmol, 1.2 equiv.) was added drop wise into the reaction flask for over 10 minutes. The resulting reaction mixture was stirred for 4 h at room temperature. Then the reaction was placed in an ice bath and was quenched by the slow addition of ice-cold water, resulting in a separation of diethyl ether layer and aqueous layer. The aqueous layer was extracted with diethyl ether (3 x 100 mL). The combined diethyl ether layer was washed with H\textsubscript{2}O and brine and then dried over MgSO\textsubscript{4}. After the MgSO\textsubscript{4} was filtered off, the solvent was removed in vacuo, and the product was obtained as a light yellow oil (12.0 g, 70% yield). This material was carried on without further purification. \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \(\delta\) 5.82 (dt, \(J = 15.1, 6.2\) Hz, 1H), 5.68 (dtt, \(J = 15.1, 7.6, 1.4\) Hz, 1H), 3.95 (d, \(J = 7.5\) Hz, 1H), 2.09 (p, \(J = 7.1\) Hz, 1H), 1.00 (t, \(J = 7.5\) Hz, 1H). \textsuperscript{13}C NMR (101 MHz, CDCl\textsubscript{3}) \(\delta\) 138.05, 125.40, 34.71, 25.14, 13.05 ppm.
Figure S1. $^1$H NMR spectrum (400 MHz in CDCl$_3$) of compound 2.
(E)-(3-Phthalimidomethyl-4-ethyl)azetidin-2-one (3): To an oven-dried round-bottom flask was added DMF (150 mL) and compound 2 (12.0 g, 80.5 mmol, 1.0 equiv.). Then phthalimide potassium salt (18.6 g, 100 mmol, 1.24 equiv.) was added to the mixture, and the reaction suspension was heated to 60 °C and stirred overnight. On the next day, the reaction mixture was added to approximately 200 mL of ice, and the mixture was stirred until all ice melted. The mixture was extracted with dichloromethane (3 x 100 mL). The combined dichloromethane layer was washed with H2O (100 mL x 2) and brine (100 mL x 4) and dried over MgSO4. After the removal of the MgSO4 by filtration, dichloromethane was removed in vacuo, and the product was obtained as a yellow solid (14.2 g, 82% yield). The product was used without further purification. 1H NMR (400 MHz, CDCl3) δ 7.85 (dd, J = 5.4, 3.0 Hz, 2H), 7.71 (dd, J = 5.4, 3.1 Hz, 2H), 5.79 (dt, J = 15.3, 6.2 Hz, 1H), 5.50 (dtt, J = 15.4, 6.2, 1.6 Hz, 1H), 4.24 (dd, J = 6.2, 1.1 Hz, 2H), 2.13 – 1.91 (p, 2H), 0.96 (t, J = 7.4 Hz, 3H). 13C NMR (101 MHz, CDCl3) δ 168.04, 136.60, 133.85, 132.22, 123.82, 120.89, 39.57, 25.78, 13.10. ESI-MS: m/z calculated for C13H14NO2 (M+H)+ 216.1025, found 216.1019.
Figure S3. $^1$H NMR spectrum (400 MHz in CDCl$_3$) of compound 3.
(±)-2-[[[(trans)-2-Ethyl-4-oxo-3-azetidinyl]ethyl]-1H-isoindole-1,3(2H)-dione (4): To an oven-dried round-bottom flask was added compound 3 (16.4 g, 76.2 mmol 1.0 equiv.) and anhydrous dichloromethane (100 mL). The solution was kept under N₂ atmosphere. N-chlorosulfonyl isocyanate (CSI, 10.0 mL, 114 mmol, 1.5 equiv.) was added dropwise, and then the reaction mixture was heated to reflux and stirred for 6 days. Reaction progress was monitored by ¹H NMR analysis of small aliquots removed from the reaction vessel. After 6 days, the reaction mixture was cooled and added to an aqueous solution of Na₂SO₃ and NaH₂PO₄, and the pH of the mixture was adjusted to ≥ 8 with NaOH. The resulting mixture was stirred overnight. Then the organic layer was separated, and the aqueous layer was extracted with dichloromethane (3 x 100 mL). The combined dichloromethane layer was washed with H₂O and brine and was dried over MgSO₄. After the removal of MgSO₄ via filtration, dichloromethane was removed in vacuo, and a yellow solid was obtained. Silica column chromatography eluting with 50:50 hexanes:ethyl acetate and then pure ethyl acetate yielded the product as a light yellow solid (2.1 g, 11% yield). ¹H NMR (400 MHz, CDCl₃) δ 7.87 (dd, J = 5.4, 3.1 Hz, 2H), 7.74 (dd, J = 5.4, 3.1 Hz, 2H), 5.93 (s, 1H) 4.12 (dd, J = 14.1, 5.6 Hz, 1H), 4.00 (dd, J = 14.1, 9.9 Hz, 1H), 3.57 (ddd, J = 8.0, 6.3, 2.2 Hz, 1H), 3.20 (ddt, J = 9.2, 5.4, 1.6 Hz, 1H), 1.74 – 1.43 (m, 2H), 0.79 (t, J = 7.4 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 168.02, 166.99, 134.17, 131.86, 123.46, 55.59, 55.14, 36.57, 27.72. ESI-MS: m/z calculated for C₁₄H₁₄N₂O₃, (M+H)⁺ 259.1079, found 259.1075.
Figure S5. $^1$H NMR spectrum (400 MHz in CDCl$_3$) of compound 4.
**Figure S6.** $^{13}$C NMR spectrum (126 MHz in CDCl$_3$) of compound 4.

Compound 4 crystallized upon standing in the CDCl$_3$ solution in an NMR tube. The crystal structure confirmed the trans configuration of side chains.

**Figure S7.** Crystal structure for one of the enantiomers for compound 4. (CCDC No.: 2033850)
(±)-trans-(3-N-(tert-Butoxycarbonyl)aminomethyl-4-ethyl)azetidin-2-one, ME-trans β, (5): To a stirred suspension of compound 4 (1.5 g, 5.81 mmol, 1.0 equiv.) in methanol (20 mL) was added anhydrous hydrazine (0.55 mL, 17.4 mmol, 3.0 equiv.) drop wise under N₂. The resulting mixture was stirred at room temperature for about 16 h. The suspension turned clear in the first few hours and then turned into a slurry. The slurry was diluted with copious methanol, and the precipitate was filtered off. Methanol in the filtrate was removed in vacuo. The resulting solid was suspended in methanol (55 mL), and then Boc₂O (5.08 g, 23.2 mmol, 4.0 equiv.) and triethylamine (3.25 mL, 23.2 mmol, 4.0 equiv.) were added. The reaction mixture was heated to reflux with stirring for 2 h. After the mixture had cooled, the solvent was removed in vacuo, and the residue was diluted with ethyl acetate (100 mL). The ethyl acetate solution was washed with 1 N HCl solution (50 mL x 2), followed by 1 N NaOH solution (50 mL x 2) and brine (50 mL). The ethyl acetate solution was dried over MgSO₄, filtered and concentrated in vacuo. The crude product was purified by column chromatography, eluting with 50:50 hexanes:ethyl acetate followed by pure ethyl acetate. An off-white solid was collected (0.782 g, 59% yield). ¹H NMR, (400 MHz, CDCl₃) δ 5.98 (s, 1H), 4.93 (s, 1H), 3.55 (m, 1H), 3.49 – 3.39 (m, 2H), 2.92 (td, J = 6.4, 2.3 Hz, 1H), 1.73 – 1.53 (m, 2H), 1.44 (s, 9H), 0.96 (t, J = 7.4 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 168.96, 155.99, 79.56, 56.49, 54.19, 38.61, 28.33, 27.61, 10.19. ESI-MS: m/z calculated for C₁₁H₂₀N₂O₃ (M+H)+ 229.1547, found 229.1539.

![Figure S8](image-url) Figure S8. ¹H NMR spectrum (400 MHz, CDCl₃) of compound 5.
Figure S9. $^{13}$C NMR spectrum (101 MHz, CDCl$_3$) of compound 5.

**ME-cis β Synthesis**

\[ 	ext{OH} \rightarrow \text{PBr}_3, \text{Et}_2\text{O, r.t.} \rightarrow \text{Br} \rightarrow \text{DMF, 60 °C} \rightarrow \]

\[ 6 \rightarrow 7 \rightarrow 8 \]

1). CSI, DCM, reflux
2). Na$_2$SO$_3$, Na$_2$HPO$_4$, H$_2$O

\[ \text{HN} \rightarrow \text{NHBoc} \]

\[ 9 \rightarrow 10 \]

1). H$_2$N-NH$_2$, MeOH
2). Boc$_2$O, Et$_3$N, MeOH

**Scheme S2.** Synthesis of the β-lactam (compound 10) that generates the ME-cis subunit in nylon-3 polymers.
(Z)-1-Bromo-2-pentene (7): Following the same procedure described above for synthesizing compound 2, compound 7 was prepared from the corresponding alcohol. Briefly, with compound 6 (10.0 g, 116 mmol, 1.0 equiv.) reacting with phosphorus tribromide (13.1 mL, 139 mmol, 1.25 equiv.) in anhydrous diethyl ether (200 mL) under N₂ atmosphere, compound 7 was obtained as a light yellow oil (9.3 g, 62.4 mmol, 54% yield). This material was carried on without purification. ¹H NMR (400 MHz, CDCl₃) δ 5.70 (dtt, J = 10.8, 7.9, 1.4 Hz, 1H), 5.60 (dt, J = 10.6, 7.3 Hz, 1H), 4.00 (d, J = 8.2 Hz, 2H), 2.16 (pd, J = 7.4 Hz, 2H), 1.02 (t, J = 7.6 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 137.55, 124.64, 27.19, 20.25, 13.68.

Because the starting alcohol is slightly impure as obtained from the supplier, the product often contains a small proportion of the E-isomer. The synthesis was carried on with this slightly impure product. However, only the desired stereoisomer the final β-lactam is obtained at the end of the reaction sequence.

Figure S10. ¹H NMR spectrum (400 MHz, CDCl₃) of compound 7. Very small resonances indicate a minor amount of the trans isomer.
**Figure S11.** $^{13}$C NMR spectrum (101 MHz, CDCl$_3$) of compound 7. Very small resonances indicate a minor amount of the *trans* isomer.

(Z)-(3-Phthalimidomethyl-4-ethyl)azetidin-2-one (8): Following the same procedure described above for synthesizing compound 3, compound 8 was prepared from 7 (13.2 g combined, 88.6 mmol, 1.0 equiv.) and phthalimide potassium salt (20.5 g, 111 mmol, 1.25 equiv.) in DMF (150 mL). After the overnight reaction, the reaction mixture was diluted with diethyl ether (350 mL). Then the organic layer was washed with water (100 mL x 2) and brine (100 mL x 4). The organic layer was dried over MgSO$_4$, filtered and concentrated *in vacuo*, generating the product as a yellow oil (17.1 g, 79.5 mmol, 90% yield). This material was carried on without purification. $^1$H NMR (400 MHz, CDCl$_3$) δ 7.84 (dd, J = 5.4, 3.1 Hz, 2H), 7.71 (dd, J = 5.5, 3.1 Hz, 2H), 5.59 (dtt, J = 10.3, 7.4, 1.4 Hz, 1H), 5.43 (dtt, J = 10.4, 7.1, 1.6 Hz, 1H), 4.32 (dd, J = 7.2, 0.9 Hz, 2H), 2.28 (pd, J = 7.5, 1.6 Hz, 2H), 1.04 (t, J = 7.5 Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 167.90, 136.10, 133.77, 132.15, 123.08, 122.14, 41.85, 34.65, 20.60, 13.97. ESI-MS calculated m/z for C$_{13}$H$_{14}$NO$_2$ (M+H)$^+$ 216.1025, found 216.1019.
Figure S12. $^1$H NMR spectrum (400 MHz, CDCl$_3$) of compound 8. Very small resonances indicate a minor amount of the trans isomer.
Figure S13. $^{13}$C NMR spectrum (101 MHz, CDCl$_3$) of compound 8. Very small resonances indicate a minor amount of the trans isomer.

(±)-2-[(cis)-2-Ethyl-4-oxo-3-azetidinyl]ethyl]-1H-isoindole-1,3(2H)-dione (9): The reaction and work-up procedures described above for compound 4, were used to prepare 9 from 8 (15.9 g, 73.9 mmol, 1.0 equiv.), with CSI (9.63 mL, 111 mmol, 1.5 equiv.) and anhydrous dichloromethane (75 mL). To the crude product of compound 9 dissolved in dichloromethane was added copious hexanes, which caused a white solid to slowly precipitate. The precipitate was isolated, washed with cold hexanes and dried in vacuo (4.70 g, 18.2 mmol, 25% yield). $^1$H NMR, (400 MHz, Chloroform-d) δ 7.86 (dd, J = 5.5, 3.1 Hz, 2H), 7.72 (dd, J = 5.5, 3.1 Hz, 2H), 6.23 (s, 1H), 4.11 (dd, J = 14.0, 8.6 Hz, 1H), 3.93 (dd, J = 13.9, 8.0 Hz, 1H), 3.81 (tdd, J = 8.0, 6.2, 1.8 Hz, 1H), 3.67 (dt, J = 9.1, 5.2 Hz, 1H), 1.79 – 1.62 (m, 2H), 0.99 (t, J = 7.4 Hz, 3H). $^{13}$C NMR (101 MHz, CDCl3) δ δ 168.25, 167.95, 134.02, 131.98, 123.40, 53.28, 50.28, 33.50, 24.03, 10.97. ESI-MS: calculated m/z for C$_{14}$H$_{14}$N$_2$O$_3$, (M+H)$^+$ 259.1077, found 259.1077.
Figure S14. $^1$H NMR spectrum (400 MHz, CDCl$_3$) of compound 9.
Compound 9 crystallized upon slow evaporation of a dichloromethane solution. The structure confirmed the expected cis configuration.
(±)-cis-(3-N-(tert-Butoxycarbonyl)aminomethyl-4-ethyl)azetidin-2-one, ME-cis β, (10): To a stirred suspension of compound 9 (800 mg, 3.1 mmol, 1 equiv.) in methanol (6.2 mL) was added anhydrous hydrazine (147 µL, 4.7 mmol, 1.5 equiv.) drop wise. It is important not to use more than 2 equivalents of hydrazine, since excessive hydrazine could result in the ring-opening for this β-lactam (there is no such problem for the trans isomer). Following the same procedure for synthesizing and purifying compound 5, compound 10 was obtained as an off-white glutinous solid (370 mg, 1.6 mmol, 52% yield). 

$^1$H NMR (500 MHz, CDCl$_3$) δ 5.98 (s, 1H), 4.96 (s, 1H), 3.67 – 3.63 (m, 2H), 3.37 – 3.29 (m, 2H), 1.68 (tq, J = 12.8, 7.5 Hz, 1H), 1.55 (ddt, J = 16.3, 14.4, 7.4 Hz, 1H), 1.44 (s, 9H), 0.97 (t, J = 7.4 Hz, 3H). $^{13}$C NMR, (126 MHz, CDCl$_3$) δ 169.89, 155.76, 79.60, 53.22, 52.13, 36.46, 28.36, 23.88, 10.96. ESI-MS: m/z calculated for C$_{11}$H$_{20}$N$_2$O$_3$ (M+H)$^+$ 229.1547, found 229.1546.

Figure S17. $^1$H NMR spectrum (500 MHz, CDCl$_3$) of compound 10.
**UPLC for Hydrophobicity Characterization of β-Lactam Monomers**

UPLC was used for determining the relative polarities of β-lactam monomers. These studies involved a Waters BEH C18 column (1.7 µm particle size, 2.1 × 50 mm), with a solvent gradient from 10% to 50% acetonitrile in water, with 0.1% (v/v) trifluoroacetic acid in both solvents. The detection was based on UV absorbance at 210 nm. The polarities of monomers should be correlated with the retention times (RT) on UPLC chromatograms: the larger the RT, the higher the net hydrophobicity.

Figure S18. $^{13}$C NMR spectrum (126 MHz, CDCl$_3$) of compound 10.
Figure S19: UPLC chromatograms with a C18 column and a solvent gradient of 10% to 50% acetonitrile in water for ME-cis β (a), ME-trans β (b), MM β (c), and NM β (d) monomers. ME-cis β and ME-trans β have a retention time (RT) of around 4.000 minutes; MM β has an RT of 3.251 minutes, and NM β has an RT of 2.742 minutes. The trend among the four β-lactams suggests that RT in these UPLC measurements provides a qualitative indicator of net polarity.

The ME-cis and ME-trans β-lactam monomers show nearly identical UPLC behavior, as shown in Figure S19a and S19b. β-Lactam monomers with one fewer CH$_2$ group (MM β, Figure S19c) or two fewer CH$_2$ groups (NM β, Figure S19d) relative to ME-cis/ME-trans β-lactams eluted more rapidly than ME-cis/ME-trans β-lactams on the UPLC, as expected for molecules that have lower net hydrophobicity but the same complement of polar groups (one amide and one urethane). Collectively, these UPLC chromatograms
suggest that ME-trans and ME-cis β-lactam monomers have very similar net hydrophobicity. These observations support the hypothesis that ME-trans and ME-cis subunits should contribute equally to the net hydrophobicity of nylon-3 polymer chains.

**Nylon-3 Polymer Synthesis**

Nylon-3 polymers were prepared in a nitrogen-purged glove box at room temperature using previously reported methods. Briefly, polymers were synthesized in either tetrahydrofuran (THF) or dimethylacetamide (DMAc). The entire quantity of β-lactam monomers was added to the reaction vessel before initiation of the copolymerization reaction. To the β-lactam solution was added a solution of the co-initiator precursor 4-tert-butylbenzoyl chloride in THF or DMAc; the amount of the co-initiator depended on the intended degree of polymerization. For instance, for an average chain length of 20, 0.05 equivalents of the co-initiator relative to the total quantity of β-lactam monomers was added. Then the solution of lithium bis(trimethylsilyl)amide in THF or DMAc (2.5 equivalents of this base relative to the amount of 4-tert-butylbenzoyl chloride) was added, and the mixture was stirred overnight at room temperature. Polymers were isolated and purified by precipitation with pentane. Side chain groups were deprotected using neat trifluoroacetic acid (TFA) with 5% (v/v) of triisopropylsilane (TIPS) relative to TFA. Copolymers at the protected stage (with Boc groups on the side chain nitrogens) were analyzed by gel permeation chromatography (GPC) using THF or DMAc as the mobile phase. Side-chain *deprotected* copolymers were characterized by proton nuclear magnetic resonance (1H NMR) spectroscopy.

THF GPC analysis involved two Waters columns (Styragel HR 4E and HR 2 particle size 5 μm) linked in series. The Waters liquid chromatography unit (Alliance) was equipped with a multangle light scattering detector (Wyatt miniDAWN TREOS, 658 nm) and a refractive index detector (Wyatt Optilab-rEX, 658 nm). THF (HPLC grade) was used as the mobile phase, with a flow rate of 1.0 mL/min at 40 °C. Number-average molecular weight (Mn) and dispersity (Đ) were calculated using ASTRA software using an estimated dn/dc value of 0.1 mL/g. DMAc GPC analysis involved two Waters styrage HR 4E columns (particle size 5 μm) linked in series on a Waters liquid chromatography instrument equipped with a refractive index detector (Waters 2410). DMAc (HPLC grade) with 10 mM LiBr was used as the mobile phase, with a flow rate of 1.0 mL/min at 80 °C. Mn and Đ were calculated using Empower software and calibration curves from polymethacrylate standards in DMAc.

The degree of polymerization (DPGPC), or average polymer chain length, was calculated based on the determined Mn value, the NMR-determined average ratio of subunits per polymer chain, and the molecular weights of the subunits using the equation below:

$$DP_{GPC} = \frac{(M_n - M_{eg})}{[M_{cationic}x + M_{hydrophobic}(1-x)]},$$
where $M_{\text{eg}}$ is the mass of the N-terminal end group (tert-butylbenzoyl), $M_{\text{cationic}}$ is the mass of the side-chain-protected cationic subunit, $M_{\text{hydrophobic}}$ is the mass of the hydrophobic subunit, and $x$ is the mole percentage of the cationic subunit determined by proton NMR.

Each deprotected polymer was dissolved in D$_2$O at a concentration of 8 mg/mL. Proton nuclear magnetic resonance ($^1$H NMR) spectra were collected on a Bruker Avance III spectrometer at 500 MHz at ambient temperature. The NMR spectra are reported in ppm using the chemical shift of the solvent as the internal standard (4.790 ppm for H$_2$O). The degree of polymerization ($D_{\text{P,NMR}}$) for each polymer was calculated from the NMR integration of proton resonances. The aromatic resonances between 7.4 and 7.7 ppm come from the N-terminal tert-butylbenzoyl group, and they were used to normalize the polymer chain with a total integral value of 4. Detailed analyses for each of the polymer NMR spectra are available for determining degrees of polymerizations and subunit compositions.
**GPC & NMR Characterizations for Nylon-3 Polymers**

The GPC chromatogram for ME-cis homopolymer could not be obtained because this polymer did not completely dissolve in DMAc, the solvent for the GPC analysis; the liquid mixture could not pass through the syringe filter, which is needed to avoid clogging the GPC column.

**Figure S20.** $^1$H NMR spectrum (500 MHz, D$_2$O) for ME-cis homopolymer.

**Calculation for DP$_{NMR}$**

$$
DP_{NMR} = (19.75 + 39.90 + 19.04 + 40.76 + 61.79) / 9 \text{ ME-cis protons} \approx 20
$$
Figure S21. GPC chromatogram for ME-trans homopolymer with Boc-protected amine groups. The mobile phase was THF, and the analysis was carried out at 40°C. The red line represents detection via light scattering (LS), and the blue line represents detection via differential refractive index (dRI). The LS peak eluting from 6 to 8 minutes comes from the column stationary phase.

| Polymer                  | $D_{GPC}$ | $M_n$  | $DP_{GPC}$ |
|--------------------------|-----------|--------|------------|
| ME-trans Homopolymer     | 1.09      | 5706   | 24         |

Calculation of $DP_{GPC}$

$$DP_{GPC}^* = \frac{(5706 - 161.22)}{228.29} \approx 24$$

*Note: 161.22 is the molecular weight of tert-butylbenzoyl group in the N-termini of polymer chains*
Figure S22. $^1$H NMR spectrum (500 MHz, D$_2$O) for ME-trans homopolymer.

Calculation for $DP_{NMR}$

$$DP_{NMR} = \frac{(21.92 + 67.45 + 46.00 + 69.37)}{9} \approx 23$$
Figure S23. GPC chromatogram for the ME-cis:ME-trans copolymer generated with a 1:3 feed ratio of ME-cis to ME-trans β-lactams, with Boc-protected side chains on the polymer. The mobile phase was THF, and the analysis was carried out at 40°C. The red line represents detection via light scattering (LS), and the blue line represents detection via differential refractive index (dRI). The LS peak eluting from 10 to 12 minutes comes from the column.

| Polymer       | $D_{GPC}$ | $M_n$ | $D_{GPC}$ |
|---------------|-----------|-------|-----------|
| 1:3 ME-cis:ME-trans | 1.16      | 4294  | 18        |

$D_{GPC} = \frac{(4294 - 161.22)}{228.29} \approx 18$
Figure S24. $^1$H NMR spectrum (500 MHz, D$_2$O) for the ME-cis:ME-trans copolymer generated with a 1:3 feed ratio of ME-cis to ME-trans $\beta$-lactams.

Due to the similarity in the chemical shifts between ME-cis and ME-trans subunits, the subunit ratio for this polymer cannot be determined by NMR. For this polymer, $x$ represents the percentage of incorporation for ME-cis subunit.

Calculation for DP$_{\text{NMR}}$

$$\text{DP}_{\text{NMR}} = \frac{(26.14 + 84.00 + 55.92 + 96.25)}{9 \text{ ME protons}} \approx 29$$
Figure S25. GPC chromatogram for the ME-cis:ME-trans copolymer generated with a 1:1 feed ratio of ME-cis to ME-trans β-lactams, with Boc-protected side chains on the polymer. The mobile phase was THF, and the analysis was carried out at 40°C. The red line represents detection via light scattering (LS), and the blue line represents detection via differential refractive index (dRI). The LS peak eluting from 6 to 8 minutes comes from the column stationary phase.

| Polymer          | $D_{GPC}$ | $M_n$  | $DP_{GPC}$ |
|------------------|-----------|--------|------------|
| 1:1-ME-cis:ME-trans | 1.06     | 5061   | 21         |

\[
DP_{GPC} = \frac{(5061 - 161.22)}{228.29} \approx 21
\]
Figure S26. $^1$H NMR spectrum (500 MHz, D$_2$O) for the ME-cis:ME-trans copolymer generated with a 1:1 feed ratio of ME-cis to ME-trans β-lactams.

Due to the similarity in the chemical shifts between ME-cis and ME-trans subunits, the subunit ratio for this polymer cannot be determined by NMR. For this polymer, \( y \) represents the percentage of incorporation for ME-cis subunit.

Calculation for $D_{\text{NMR}}$

\[
D_{\text{NMR}} = \frac{(20.59 + 61.51 + 41.04 + 68.35)}{9 \text{ ME protons}} \approx 21
\]
**Figure S27.** GPC chromatogram for the ME-cis:ME-trans copolymer generated with a 3:1 feed ratio of ME-cis to ME-trans β-lactams, with Boc-protected side chains on the polymer. The mobile phase was DMAc, and the analysis was carried out at 80°C. The detection was via refractive index. The spike from 19 to 22 minutes came from water in the DMAc solvent.

| Polymer                  | $D_{GPC}$ | $M_n$  | $DP_{GPC}$ |
|--------------------------|-----------|--------|------------|
| 3:1-ME-cis:ME-trans      | 1.22      | 5184   | 22         |

$$DP_{GPC} = \frac{(5184 - 161.22)}{228.29} \approx 22$$
Figure S28 $^1$H NMR spectrum (500 MHz, D$_2$O) for the ME-cis:ME-trans copolymer generated with a 3:1 feed ratio of ME-cis to ME-trans $\beta$-lactams.

Due to the similarity in the chemical shifts between ME-cis and ME-trans subunits, the subunit ratio for this polymer cannot be determined by NMR. For this polymer, $z$ represents the percentage of incorporation for ME-cis subunit.

Calculation for $D_{P_{NMR}}$

$$D_{P_{NMR}} = \frac{(23.53 + 77.01 + 50.53 + 83.96)}{9 \text{ ME protons}} \approx 26$$
The GPC chromatogram for ME-cis:TM copolymer could not be obtained. The polymer did not fully dissolve in DMAc, the solvent for GPC. The mixture could not pass through the syringe filter, which is needed to avoid clogging the GPC column.

Figure S29. $^1$H NMR spectrum (500 MHz, D$_2$O) for the ME-cis:TM polymer.

Calculation for $\text{DP}_{\text{NMR}}$

$14.22 = 1$ ME-cis proton; $44.34 = 3$ ME-cis protons;
$81.52 + 47.64 = 5$ ME-cis protons + 12 TM protons + 9 Initiator protons;
$\text{ME-cis} \approx 15$ and $\text{TM} \approx 4$
$\text{DP}_{\text{NMR}} = \text{ME-cis} + \text{TM} = 19$
Figure S30. GPC chromatogram for the ME-trans:TM polymer with Boc-protected side chains. The mobile phase was THF, and the analysis was carried out at 40 °C. The red line represents detection via light scattering (LS), and the blue line represents detection via differential refractive index (dRI). The LS peak eluting from 6 to 8 minutes comes from the column stationary phase.

| Polymer     | $D_{\text{GPC}}$ | Mn     | $D_{\text{GPC}}$ |
|-------------|------------------|--------|------------------|
| ME-trans:TM | 1.14             | 3130   | 14               |

$$D_{\text{GPC}} = (3130 - 161.22) / [(228.28 \times 0.79) + (127.19 \times 0.21)] \approx 14$$
Figure S31. $^1$H NMR spectrum (500 MHz, D$_2$O) for the ME-trans:TM polymer.

Calculation for DP$_{NMR}$

14.82 = 1 ME-trans proton; 45.19 = 3 ME-trans protons; 78.90 + 53.96 = 5 ME-trans protons + 12 TM protons + 9 Initiator protons; ME-trans $\approx$ 15 and TM $\approx$ 3

DP$_{NMR}$ = ME-trans + TM = 18
Figure S32. GPC chromatogram for the ME-cis:DMCH polymer with Boc-protected side chains. The mobile phase was THF, and the analysis was carried out at 40°C. The red line represents detection via light scattering (LS), and the blue line represents detection via differential refractive index (dRI). The LS peak eluting from 6 to 8 minutes comes from the column stationary phase.

| Polymer       | $D_{GPC}$ | $M_n$ | $DP_{GPC}$ |
|---------------|-----------|-------|------------|
| ME-cis:DMCH   | 1.15      | 3498  | 16         |

$DP_{GPC} = (3130 - 161.22) / [(228.28 \times 0.79) + (153.23 \times 0.21)] \approx 16$
Figure S33. $^1$H NMR spectrum (500 MHz, D$_2$O) for the ME-cis:DMCH polymer.

**Calculation for DP$_{NMR}$**

14.56 = 1 ME-cis proton; 44.79 = 3 ME-cis protons; 
91.98 + 46.57 = 5 ME-cis protons + 14 DMCH protons + 9 Initiator protons; 
ME-cis $\approx$ 15 and DMCH $\approx$ 4

$\text{DP}_{NMR} = \text{ME-cis} + \text{DMCH} = 19$
Figure S34. GPC chromatogram for the ME-trans:DMCH polymer with Boc-protected side chains. The mobile phase was THF, and the analysis was carried out at 40°C. The red line represents detection via light scattering (LS), and the blue line represents detection via differential refractive index (dRI). The LS peak eluting from 6 to 8 minutes comes from the column stationary phase.

| Polymer         | $D_{GPC}$ | $M_n$ | $DP_{GPC}$ |
|-----------------|-----------|-------|------------|
| ME-trans:DMCH   | 1.14      | 3226  | 15         |

$$DP_{GPC} = \frac{(3226 - 161.22)}{[(228.28 \times 0.77) + (153.23 \times 0.23)]} \approx 15$$
Figure S35. $^1$H NMR spectrum (500 MHz, D$_2$O) for the ME-trans:DMCH polymer.

Calculation for $\text{DP}_{\text{NMR}}$

- $16.14 = 1$ ME-trans proton; $52.69 = 3$ ME-trans protons;
- $84.21 + 76.41 = 5$ ME-trans protons + 14 DMCH protons + 9 Initiator protons;

ME-trans $\approx 17$ and DMCH $= 5$

$\text{DP}_{\text{NMR}} = \text{ME-trans} + \text{DMCH} = 22$
Assays for Nylon-3 Polymers

Bacteria Inhibitory Assay Protocol
Nylon-3 polymers were tested against *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Enterococcus faecium*. For these experiments, *E. coli* was cultured in (lysogeny broth) LB medium or EZ Rich Defined Medium (EZRDM);<sup>2,3</sup> *B. subtilis* and *S. aureus* were cultured in LB medium; *E. faecium* was cultured in brain heart infusion (BHI) medium. For *E. coli*, *B. subtilis* and *S. aureus*, each bacterium was cultured in the medium at 37°C with shaking for 12 h. *E. faecium* was cultured with the same method for 18 h. After incubation, the bacteria suspensions were diluted to 20 Klett. In a clear, sterile polystyrene 96-well plate, a two-fold serial dilution of each polymer in the culture medium (with a starting concentration of 200 or 250 µg/mL) was combined with an equal volume of the diluted bacterial suspension, resulting in a total volume of 200 µL in each well. The plates were incubated at 37°C for 6 h. We define MIC as the lowest concentration for which no cell growth could be detected as determined by optical density (OD) at 590 nm. The OD for the growth medium defines 0% relative OD; the OD for bacteria in growth medium with no polymer or drug added defines 100% relative OD.

Relative OD (%) = \( \frac{(OD_{\text{Experimental}} - OD_{\text{Medium}})}{(OD_{\text{Bacteria}} - OD_{\text{Medium}})} \times 100\% \)
Figure S36. Assays for inhibition of bacterial growth by copolymers containing either ME-trans or ME-cis: (a) *E. coli* in LB, (b) *E. coli* in EZRDM, (c) *S. aureus* in LB, (d) *B. subtilis* in LB, and (e) *E. faecium* in BHI. For the vertical axis, 100% OD590 is defined by OD590 of bacteria in medium in the absence of polymer, and 0% OD590 is defined by OD590 of growth medium alone. The MIC was assigned based on the lowest concentration at which OD590 reached its minimum. For measurements in complex growth media (LB or BHI), OD590 increases relative to the MIC at higher concentrations, a trend that we attribute to aggregation caused by interactions between the cationic polymers and polyanions in the complex media.2,3 In EZRDM, a chemically defined medium that does not contain polyanions, no increase in OD590 is observed as polymer concentration increases above the MIC; this trend supports our explanation of the increases in OD590 at high polymer concentrations in the complex media.
Figure S37. Assays for inhibition of bacterial growth by the ME-trans or ME-cis homopolymers (each heterochiral): (a) *E. coli* in LB, (b) *E. coli* in EZRDM, (c) *S. aureus* in LB, (d) *B. subtilis* in LB, and (e) *E. faecium* in BHI. For the vertical axis, 100% OD590 is defined by OD590 of bacteria in medium in the absence of polymer, and 0% OD590 is defined by OD590 of growth medium alone. The MIC was assigned based on the lowest concentration at which OD590 nm reached its minimum. For measurements in complex growth media (LB or BHI), OD590 increases relative to the MIC at higher concentrations, a trend that we attribute to aggregation caused by interactions between the cationic polymers and polyanions in the complex media. In EZRDM, a chemically defined medium that does not contain polyanions, no increase in OD590 is observed as polymer concentration increases above the MIC; this trend supports our explanation of the increases in OD590 at high polymer concentrations in the complex media.
**Table S1.** Bacteria inhibitory results for ME-cis and ME-trans homopolymers.

| Polymer                  | E. coli (LB) | E. coli (EZRDM) | B. subtilis (LB) | S. aureus (LB) | E. faecium (BHI) |
|--------------------------|--------------|-----------------|-----------------|----------------|-----------------|
| ME-cis Homopolymer       | > 200        | 125             | > 200           | 50             | > 200           |
| ME-trans Homopolymer     | > 200        | 62.5            | > 200           | 50             | > 200           |

aMIC: minimum inhibitory concentration  
bLB: lysogeny broth  
cEZRDM: EZ rich defined medium  
dBHI: brain heart infusion

**Hemolysis Assay Protocol**

Expired human blood was obtained from UW-Madison hospital. Human red blood cells (RBCs) were isolated by centrifugation and washed to remove dead cells by several rounds of suspension in TBS buffer followed by isolation via centrifugation at 3500 rpm, 4 °C, until the supernatant was clear and colorless.

A 2% (v/v) suspension of human RBCs was prepared in TBS buffer. Two-fold serial dilutions of polymer solutions in TBS buffer were prepared in tissue-culture treated sterile polystyrene 96-well plates, with each well containing 100 µL of polymer solution. Then 100 µL of 2% RBC suspension in TBS buffer was added to each well of the plate. The plate was incubated at 37 °C for 1 hr, and then the cells were pelleted in the plates by centrifugation at 3500 rpm for 5 min. After the centrifugation, 80 µL of supernatant from each well was transferred to another black-walled, clear-bottom, sterile polystyrene 96-well plate, and the absorbance at 405 nm was recorded. For wells with absorbance larger than 1.0, TBS buffer was used to dilute the supernatant so that absorbance was below 1.0. For these wells, the absorbance value below 1.0 was multiplied by the dilution factor before determining the concentrations of hemoglobin. The average absorbance of cells incubated with 10% (v/v) Triton-X 100 dissolved in TBS buffer defines 100% hemolysis; the average absorbance of cells incubated with TBS buffer alone defines 0% hemolysis.

\[
\text{Hemolysis} \% = \frac{\text{Abs}_{\text{experimental}} - \text{Abs}_{\text{TBS}}}{\text{Abs}_{\text{Triton-X 100}} - \text{Abs}_{\text{TBS}}} \times 100\%
\]

**Aggregation Studies**

Critical micelle concentrations (CMC values) were determined with a previously reported method. Specifically, 1,6-diphenyl-1,3,5-hexatriene (DPH, 2.9 mg, 12.5 µmol) dye was dissolved in 5 mL of THF and diluted to a total volume of 10 mL with millipore water. Then 250 µL of this DPH dye solution was diluted to 1 mL in Tris-buffered saline (TBS) buffer solution. After that, 500 µL of this solution was diluted further, to a total volume of 25 mL in TBS buffer (final [DPH] = 6.2 µM). Nylon-3 polymers were subjected to 2-fold serial dilutions in TBS buffer, ranging from 1 mg/mL to 7.8 µg/mL; n-dodecyl β-D-maltoside
(DDM detergent) was prepared in ultrapure water at a concentration of 10 mM as a positive control. Serially diluted nylon-3 polymer solutions and DDM solutions were added to a black polypropylene 96-well plate, with a total volume of 50 µL in each well. A 50 µL aliquot of the DPH solution was then added to each of the wells with polymer or detergent solutions. The 96-well plate was incubated under dark condition for 1 hour at 50°C, and the fluorescence intensity was measured using a Biotek Synergy 2 Microplate reader (excitation 355 nm, emission 460 nm) afterwards. The fluorescence intensity of each sample was plotted against the log of the sample concentration. For the conventional detergent DDM, the critical micelle concentration (CMC) was determined from the intersection of the two lines formed by linear regression calculated for the concentrations that show low fluorescence and those that show high fluorescence. None of the polymers studied here showed significant DPH solubilization up to 500 µg/mL (Figure S38a-b), indicating that no micellar aggregation was observed for these polymers under hemolysis experimental conditions. In contrast, DDM detergent showed significant DPH solubilization. The CMC measured here for DDM (0.190 mM) was comparable to the literature precedent (0.165 mM) (Figure S38c).5

Figure S38. Critical micelle concentration study with DPH dye for a). ME:TM polymers, b). ME:DMCH polymers, and c). DDM detergent control. Aggregation studies suggested that in TBS buffer, none of the polymers displayed micellar aggregation.

HeLa Cell Assay
HeLa cells (generously provided by Dr. James Bruce from the lab of Prof. Paul Ahlquist in the Department of Oncology at UW-Madison) were cultured in a Gibco high-glucose DMEM medium containing 10% (v/v) fetal bovine serum and 1% (v/v) penicillin-
streptomycin. The cells were passaged every 3 to 4 days. To each well of a tissue-culture treated clear bottom polystyrene 96-well plate, 5,000 cells were seeded. The cells were incubated at 37 °C for 24 h, for the cells to attach to the plate.

The CytoTox-One assay kit was purchased from Promega Inc. In a blank 96-well plate, nylon-3 polymers dissolved in PBS buffer were subjected to twofold serial dilutions, resulting in concentrations ranging from 500 to 3.9 µg/mL, with each well containing 100 µL of polymer solution. In the plate with HeLa cells, DMEM was carefully removed, and then the polymer solutions were added to the wells with cells. The plate was incubated for 2 h at 37 °C. Then 100 µL of the assay substrate was added to each well, and the plate was incubated at room temperature for 10 min. Subsequently, 50 µL of the stop solution was added to each well, and the plate was shaken for 1 min. The fluorescence signal with excitation at 560 nm and emission at 590 nm was recorded. The average fluorescence of cells incubated with Triton X 100 in PBS at 10% (v/v) defines 100% cell lysis; the average fluorescence of cells incubated with PBS buffer defines 0% cell lysis.

\[
\text{Cell lysis} \% = \frac{(\text{RFU}_{\text{experimental}} - \text{RFU}_{\text{cells}})}{(\text{RFU}_{\text{triton-x 100}} - \text{RFU}_{\text{cells}})} \times 100\%
\]

**GUV Leakage Experiment**

Lipid solutions composed of 1 mM DOPC/DOPS (9:1), labeled with 0.1 % rhodamine-PE were prepared in chloroform. Approximately 15 µL of lipid solutions in chloroform were spotted on the conductive surfaces of two ITO glass slides and dried under a stream of nitrogen gas. Once dried, the slides were placed in a lyophilizer for 1 h to remove residual chloroform.

During this hour of drying, two isosmolar buffers were prepared for GUV experiments; a) electroformation buffer and b) GUV wash/assay buffer. Isosmolarity, measured using a Wescor Vapro 5520 osmometer, is needed to maintain structural integrity of the vesicles during GUV washing. The electroformation buffer (1 mM HEPES, 250 mM sucrose) was prepared containing 1 mg/mL FITC-labeled dextran dye. The GUV wash/assay buffer was PBS, prepared by dissolving one PBS tablet in 200 mL millipore water, followed by sterile-filtration.

After the 1 h of lyophilization, greased O-rings were carefully placed on the glass ITO-coated surfaces, to create a chamber around the dried lipids. Then the dye-containing electroformation solution was added within the O-rings. The O-rings with dye solutions were sandwiched between two ITO-slides, with the conductive sides touching O-rings; the two glass slides were then clamped and put under a 3 V sine wave of alternating current with a frequency of 10 Hz using a Siglent SDG1010 function generator and incubated at 37 °C for 2 h. Then the frequency was reduced to 0.5 Hz for another 5 min to aid in the release of vesicles from the glass surface. After electroformation, the glass slides were carefully separated, and the GUV suspensions were collected.
Figure S39. 3 V, 10 Hz alternating current enables the formation of GUVs.

To remove unencapsulated dye, the GUV suspensions were washed with PBS using PET membrane filters (Corning) with 3 µm pores. The filtration was kept running until the GUV suspension became colorless. Alternatively, dye that was not incapsulated could be removed by centrifugation in the PET membrane filter at 500 x g for 30 sec twice.

To a BSA-passivated 96-well glass plate was added 100 µL of GUV suspension in PBS buffer. Alternatively, 100 µL of GUV suspension in PBS was added to a polystyrene 96-well plate with ultra-low attachment hydrogel coating (Corning). The plate was placed on a Zeiss 880 LSM microscope for observing the vesicle morphologies. Then polymer solutions were added according to the desired concentrations. Effects of the polymers on vesicles were recorded with the microscope.

| Name         | Average MW | Average Stokes Radii |
|--------------|------------|----------------------|
| FD4          | 4 kDa      | 14 Å                 |
| FD10S        | 10 kDa     | 23 Å                 |
| FD20S        | 20 kDa     | 32 Å                 |
| FD40S        | 40 kDa     | 45 Å                 |
| TRITC1287-Dextran | 155 kDa   | 85 Å                 |

Table S2. Dextran dye information for vesicle studies, with average molecular weight and average Stokes radii provided by Sigma Aldrich.

**Black Lipid Membrane Experiments**

Black lipid membrane (BLM) experiments were conducted by forming a planar lipid bilayer across a 150 µm aperture in a polystyrene cup. For this, 38 mM DOPC/DOPS (9:1) lipid in n-decane was painted across the cup aperture and allowed to dry. After 15 min, the cup was placed within a BLM chamber, and buffer was added to both sides of the cup aperture. One side received 25 mM HEPES, 100 mM KCl; the other received 25 mM
HEPES, 10 mM KCl. When a pore forms, this asymmetric salt condition provides the means to generate a current between the two sides. After adding the buffers, the lipid in n-decane was again painted across the aperture to form a lipid/n-decane mixture that seals the cup aperture. Excess n-decane was then removed by bubbling the aperture to allow for lipid bilayer formation. In this experiment, 2 μL of 250 μg/mL ME-cis:TM polymer solution in the HEPES buffer with 100 mM KCl was added to one side of the bilayer while monitoring for the passage of current across the membrane.

**LUV Leakage Assays**
A 1 mM lipid mixture solution of DOPC/DOPS (9:1) in chloroform was added to a glass test tube; chloroform was evaporated under a stream of N₂ gas. Then the lipid film was placed in a lyophilizer for 1 h. The lipid was hydrated with PBS containing equimolar FITC-labeled dextran and the 155 kDa TRITC-labeled dextran solution. The resulting suspensions were subsequently frozen with liquid nitrogen and thawed at 50 °C. Three freeze-thaw cycles were performed to fully hydrate the lipids and ensure homogenous encapsulation of the dyes. After this hydration, the liposome suspensions were loaded into one of the syringes (Figure S40b) and carefully placed into one end of the mini-extruder (Avanti Polar Lipids) (Figure S40c). The lipid-dye mixture was extruded by passage through a 200 nm filter 15 times. After the extrusion, unencapsulated FITC-labeled dextran dye was removed by centrifuging the LUV suspension at 16,000 g several times, until the supernatant became colorless.

![Figure S40](image.png)

After the dye removal, LUVs suspensions were diluted to 5 mL with PBS. The polymer solutions in PBS buffer were subjected to a two-fold serial dilution in a polystyrene 96-well plate with ultra-low attachment hydrogel coating (Corning). Then 100 μL of LUV suspension was added to each well with polymer solution or control solution, and the FITC fluorescence was measured using a Biotek Cytation 1 plate reader. The excitation wavelength was 490 nm, and the emission wavelength was 520 nm. For control experiments, 100 μg/mL of melittin solution in PBS buffer (after the addition of LUVs) defined 100% dye release; PBS buffer with LUVs defined 0% dye release.

\[
\text{Liposome dye leakage %} = \left( \frac{\text{RFU}_{\text{experimental}} - \text{RFU}_{\text{PBS}}}{\text{RFU}_{\text{Melittin}} - \text{RFU}_{\text{PBS}}} \right) \times 100\%
\]
Melittin solutions in PBS buffer from 4 mg/mL to 50 µg/mL were tested against LUVs loaded with 155 kDa TRITC-labeled dextran and 40 kDa FITC-dextran dye. All of these melittin solutions resulted in similar fluorescence signals (Figure S42). Thus, 200 µg/mL solution was used as a positive control for the dye leakage experiments, resulting in a solution with a concentration 100 µg/mL after the addition of LUV suspension.
Figure S42. Melittin solution in PBS buffer tested against LUVs loaded with 155 kDa TRITC-labeled dextran and 40 kDa FITC-labeled dextran. 0 µg/mL came from PBS buffer control, with liposomes but no melittin.

Similar to the LUV leakage experiments with 9:1-DOPC:DOPS lipid composition, LUV leakage experiments with LUVs prepared with 63% DOPC, 7% DOPS, and 30% cholesterol were performed to observe the effect of cholesterol on LUV leakages induced by nylon-3 polymers. Figure S43 suggests that incorporation of 30% cholesterol does not cause a significant difference in dye leakage activities induced by polymers, relative to a lack of cholesterol.
Figure S43. FITC-dextran dye leakage activities for 63:7:30-DOPC:DOPS:cholesterol LUVs induced by a). ME-cis:TM; b). ME-trans:TM; c). ME-cis:DMCH; and d). ME-trans:DMCH polymer.

References
(1) Zhang, J.; Kissounko, D. A.; Lee, S. E.; Gellman, S. H.; Stahl, S. S. Access to Poly-β-Peptides with Functionalized Side Chains and End Groups via Controlled Ring-Opening Polymerization of β-Lactams. J. Am. Chem. Soc. 2009, 131 (4), 1589–1597.
(2) Neidhardt, F. C.; Bloch, P. L.; Smith, D. F. Culture Medium for Enterobacteria. J. Bacteriol. 1974, 119 (3), 736–747.
(3) Choi, H.; Chakraborty, S.; Liu, R.; Gellman, S. H.; Weisshaar, J. C. Medium Effects on Minimum Inhibitory Concentrations of Nylon-3 Polymers against E. Coli. PLoS ONE 2014, 9 (8), e104500.
(4) Chattopadhyay, A.; London, E. Fluorimetric Determination of Critical Micelle Concentration Avoiding Interference from Detergent Charge. Anal. Biochem. 1984, 139 (2), 408–412.
(5) Vanaken, T.; Foxall-Vanaken, S.; Castleman, S.; Ferguson-Miller, S. [3] Alkyl Glycoside Detergents: Synthesis and Applications to the Study of Membrane Proteins. Methods Enzymol. 1986, 125, 27–35.