

## Supporting Information

### Helical Antimicrobial Polypeptides with Radial Amphiphilicity

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## **Experimental section:**

**Materials.** All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used as received unless otherwise specified. Anhydrous dimethylformamide (DMF), ethyl acetate (EtOAc), and hexane were dried by passing them through alumina columns and kept anhydrous by storing them in the presence of molecular sieves in a glove box. Hexamethyldisilazane (HMDS) was dissolved in DMF in a glovebox. SiliaFlash P60 silica gel (particle size 40-63  $\mu\text{m}$ ) was purchased from SiliCycle Inc. (Quebec City, Quebec, Canada) and heated to 150  $^{\circ}\text{C}$  for 48 h before use. Spectra/Por<sup>®</sup> dialysis tubing with a molecular weight cut-off (MWCO) of 1 kDa was purchased from Spectrum Laboratories (Rancho Dominguez, CA, USA). Gram-negative bacteria, DH5 $\alpha$  (*Escherichia coli*), MG1655 (*E. coli*), C101 (*Pseudomonas aeruginosa*), and Gram-positive bacteria, ATCC12608 (*Staphylococcus aureus*) and ATCC11778 (*Bacillus toyonensis*) were grown in luria broth (LB) medium at 37  $^{\circ}\text{C}$ . Clinical isolated *Helicobacter pylori* strains, B107, J291, J99, J99-AF, J99-A9 and J99-A11, supplied by Dr. Peek and Dr. Chen, were incubated in brucella broth (BB) with 10% fetal bovine serum (FBS) supplemented with vancomycin (5  $\mu\text{g}/\text{mL}$ ) at 37  $^{\circ}\text{C}$ . Among them, J99-AF, J99-A9 and J99-A11 are drug resistant bacteria. All lipids were obtained from Avanti Polar Lipids, Inc. Propidium iodide (PI) and BacLight<sup>™</sup> Kit L-7012 was purchased from Thermo Fisher Scientific Inc. Artificial tears were obtained from Boss Safety Products. *Pseudomonas Aeruginosa* Elastase was purchased from Elastin Products Company, Inc. LL-37, LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLPRTES, was obtained from

AnaSpec, Inc. Elastase from human leukocytes, plasma from human, human serum was obtained from Sigma-Aldrich.

**Characterization.**  $^1\text{H}$  NMR spectra were recorded on a Varian U500 MHz or a VXR-500 MHz spectrometer.  $^{13}\text{C}$ - $^1\text{H}$  HSQC (heteronuclear single quantum coherence) NMR,  $^1\text{H}$ - $^1\text{H}$  TOCSY (total correlation spectroscopy) NMR, and NOESY (nuclear overhauser effect spectroscopy, the mixing time is 150 ms) NMR were recorded on a VNS750NB spectrometer. Chemical shifts were reported in ppm and referenced to the solvent proton impurities. The molecular weights of prepared polypeptides were determined by gel permeation chromatography (GPC) equipped with an isocratic pump (Model 1100, Agilent Technology, Santa Clara, CA, USA), a DAWN HELEOS multi-angle laser light scattering detector (MALLS) detector (Wyatt Technology, Santa Barbara, CA, USA), and an OptilabrEX refractive index detector (Wyatt Technology, Santa Barbara, CA, USA). The detection wavelength of HELEOS was set at 658 nm. Separations were performed using serially connected size exclusion columns (100 Å, 500 Å,  $10^3$  Å and  $10^4$  Å Phenogel columns, 5  $\mu\text{m}$ ,  $300 \times 7.8$  mm, Phenomenex, Torrance, CA, USA) at 60 °C using DMF containing 0.1 mol/L LiBr as the mobile phase. The MALLS detector was calibrated using pure toluene and can be used for determination of the absolute molecular weights (MWs). The MWs of polymers were determined based on the  $\text{dn}/\text{dc}$  value of each polymer sample calculated offline by using the internal calibration system processed by the ASTRA V software (version 5.1.7.3, Wyatt Technology, Santa Barbara, CA, USA).

Circular dichroism (CD) measurements were carried out on a JASCO J-815 CD spectrometer. The polypeptides samples were prepared at concentrations of 0.40 mg/mL in water, and placed in a quartz cell with a pathlength of 0.10 cm prior to measurements. Infrared spectra were recorded on a Perkin Elmer 100 serial FTIR spectrophotometer calibrated with polystyrene film. Lyophilization was conducted in a FreeZone lyophilizer (Labconco, Kansas City, MO, USA). HPLC analyses were performed on a Shimadzu CBM-20A system (Shimadzu, Kyoto, Japan) equipped with SPD20A PDA detector (190 nm-800 nm) and RF10Ax1 fluorescence detector, and an analytical C18 column (Shimadzu, 3  $\mu$ m, 50\*4.6 mm, Kyoto, Japan).

**Synthesis of  $\gamma$ -(6-chlorohexyl)-L-glutamate (CH-L-Glu).** L-Glutamic acid (10.0 g, 68.0 mmol) and 6-chlorohexanol (15 mL, 112.4mmol) were mixed and stirred at 0 °C, followed by the dropwise addition of H<sub>2</sub>SO<sub>4</sub> (4 mL). The reaction was allowed to warm up to the room temperature and kept stirred for 24 h. Saturated Na<sub>2</sub>CO<sub>3</sub> solution was then added to adjust the pH value to 7. The resulting precipitate was collected by filtration and purified by recrystallization from isopropanol/H<sub>2</sub>O (1:1, v/v). Isopropanol was removed under vacuum and water was removed via lyophilization to obtain a white solid powder (6.92 g, yield: 38%). <sup>1</sup>H NMR (DMSO:DCl-D<sub>2</sub>O, 9:1, v/v):  $\delta$  3.91 (t, 2H, -CH<sub>2</sub>OOC-), 3.82 (t, 1H,  $\alpha$ -H), 3.52 (t, 2H, -CH<sub>2</sub>Cl), 2.53-2.32 (m, 2H, -CH<sub>2</sub>CH<sub>2</sub>COO-), 1.98 (m, 2H, -CH<sub>2</sub>CH<sub>2</sub>COO-), 1.64-1.17 (m, 8H, ClCH<sub>2</sub>(CH<sub>2</sub>)<sub>4</sub>CH<sub>2</sub>O-).

CH-DL-Glu was synthesized similarly using DL-glutamic acid as the starting material (Yield: 36 %). <sup>1</sup>H NMR (DMSO:DCl-D<sub>2</sub>O, 9:1, v/v): 3.91 (t, 2H, -CH<sub>2</sub>OOC-), 3.82 (t, 1H, α-H), 3.52 (t, 2H, -CH<sub>2</sub>Cl), 2.53-2.32 (m, 2H, -CH<sub>2</sub>CH<sub>2</sub>COO-), 1.98 (m, 2H, -CH<sub>2</sub>CH<sub>2</sub>COO-), 1.64-1.17 (m, 8H, ClCH<sub>2</sub>(CH<sub>2</sub>)<sub>4</sub>CH<sub>2</sub>O-).

**Synthesis of CH-L-Glu based N-carboxyanhydride (CH-L-Glu-NCA).** A round-bottomed flask (100 mL) was charged with CH-L-Glu (7.3 g, 27.4 mmol) and dried under vacuum for 2 h. Anhydrous tetrahydrofuran (THF, 60 mL) and phosgene (15wt% in toluene, 39.2 mL, 54.9mmol) were added successively with the protection of nitrogen. The mixture was stirred at 50 °C for 2 h. The solvent was removed under vacuum to yield an oily liquid. The product was purified by silica gel column chromatography using EtOAc/hexane (from 100 % to 60 % hexanes) as the eluent (6.6 g, yield: 83 %). <sup>1</sup>H NMR (CDCl<sub>3</sub>, ppm): δ 7.16 (s, 1H, -NH), 4.43 (t, 1H, -CHNH), 4.05 (t, 2H, -CH<sub>2</sub>OOC-), 3.51 (t, 2H, -CH<sub>2</sub>Cl), 2.51 (t, 2H, -CH<sub>2</sub>CH<sub>2</sub>COO-), 2.25-2.07 (m, 2H, -CH<sub>2</sub>CH<sub>2</sub>COO-), 1.79-1.30 (m, 8H, ClCH<sub>2</sub>(CH<sub>2</sub>)<sub>4</sub>CH<sub>2</sub>O-). <sup>13</sup>C NMR(CDCl<sub>3</sub>, ppm): δ 172.8, 170.0, 152.6, 65.4, 57.1, 45.2, 32.6, 29.7, 28.5, 27.0, 26.6, 25.4.

CH-DL-Glu-NCA was synthesized similarly using CH-DL-Glu as the starting material (Yield: 79 %). <sup>1</sup>H NMR (CDCl<sub>3</sub>, ppm): δ 7.31 (s, 1H, -NH), 4.41 (s, 1H, -CHNH), 3.99 (s, 2H, -CH<sub>2</sub>OOC-), 3.45 (s, 2H, -CH<sub>2</sub>Cl), 2.45 (s, 2H, -CH<sub>2</sub>CH<sub>2</sub>COO-), 2.10 (d, 2H, -CH<sub>2</sub>CH<sub>2</sub>COO-), 1.75-1.22 (m, 8H, ClCH<sub>2</sub>(CH<sub>2</sub>)<sub>4</sub>CH<sub>2</sub>O-). <sup>13</sup>C NMR (CDCl<sub>3</sub>, ppm): δ

172.8, 170.2, 152.7, 65.3, 57.1, 45.3, 32.5, 29.6, 28.5, 27.0, 26.6, 25.3.

**Synthesis of poly( $\gamma$ -6-chlorohexyl-L-glutamate) (PCHLG).** In a glovebox, CH-L-Glu NCA (100 mg, 0.34mmol) was dissolved in DMF (1.5 mL), followed by the addition of 85  $\mu$ L or 136  $\mu$ L HMDS (0.1 M) in DMF to obtain PCHLG<sub>40</sub> and PCHLG<sub>28</sub>. The mixture was stirred at room temperature for 48 h. The polymer was then obtained by precipitation in cold methanol and dried under vacuum at 40 °C for 8 h (Yield: PCHLG<sub>40</sub>: 66 %, PCHLG<sub>28</sub>: 56%). <sup>1</sup>H NMR (CDCl<sub>3</sub>:TFA-d, 85:15, v/v): DP=40:  $\delta$  4.60 (m, 1H, -CHNH), 4.09 (m, 2H, -CH<sub>2</sub>OOC-), 3.52 (t, 2H, -CH<sub>2</sub>Cl), 2.50 (m, 2H, -CH<sub>2</sub>CH<sub>2</sub>COO-), 2.19-1.90 (m, 2H, -CH<sub>2</sub>CH<sub>2</sub>COO-), 1.81-1.30 (m, 8H, ClCH<sub>2</sub>(CH<sub>2</sub>)<sub>4</sub>CH<sub>2</sub>O-). DP=25  $\delta$  4.60 (m, 1H, -CHNH), 4.09 (m, 2H, -CH<sub>2</sub>OOC-), 3.52 (t, 2H, -CH<sub>2</sub>Cl), 2.50 (m, 2H, -CH<sub>2</sub>CH<sub>2</sub>COO-), 2.19-1.90 (m, 2H, -CH<sub>2</sub>CH<sub>2</sub>COO-), 1.81-1.30 (m, 8H, ClCH<sub>2</sub>(CH<sub>2</sub>)<sub>4</sub>CH<sub>2</sub>O-).

PCHDLG<sub>40</sub> was synthesized similarly using CH-DL-Glu-NCA as the monomer (M/I=40) (Yield: 72 %). <sup>1</sup>H NMR (CDCl<sub>3</sub>/TFA-d, 85:15, v/v):  $\delta$  4.60 (m, 1H, -CHNH), 4.10 (m, 2H, -CH<sub>2</sub>OOC-), 3.52 (t, 2H, -CH<sub>2</sub>Cl), 2.48 (s, 2H, -CH<sub>2</sub>CH<sub>2</sub>COO-), 2.28-1.90 (m, 2H, -CH<sub>2</sub>CH<sub>2</sub>COO-), 1.81-1.30 (m, 8H, ClCH<sub>2</sub>(CH<sub>2</sub>)<sub>4</sub>CH<sub>2</sub>O-).

**Synthesis of PHLG-BIm polypeptides.** PCHLG<sub>40</sub> (86.5 mg, 0.35 mmol of Cl groups) in DMF (2 mL) and NaI (157 mg, 1.05 mmol) in acetonitrile (2 mL) was mixed and added to 1-methylbenzimidazole (92.4 mg, 0.70 mmol) in a 25 mL Schlenk tube. The mixture

was stirred at 80 °C for 48 h. After most solvent was removed under vacuum, NaCl aqueous solution (1.0 M, 3 mL) was added. The solution was then stirred at room temperature for 3 h to promote ion exchange. The product was purified by dialysis (MWCO = 1 kDa) against distilled water for 3 days. White solid powder was obtained after lyophilization (yield: 62%). <sup>1</sup>H NMR (TFA-d): δ 9.02 (s, 1H, -NCHN-), 7.86 (m, 4H, Ar-H), 4.86 (s, 1H, α-H), 4.36 (m, 2H, -COOCH<sub>2</sub>(CH<sub>2</sub>)<sub>4</sub>CH<sub>2</sub>N-), 4.29 (s, 2H, -COOCH<sub>2</sub>(CH<sub>2</sub>)<sub>4</sub>CH<sub>2</sub>N-), 4.24 (s, 3H, -NCH<sub>3</sub>), 2.72 (s, 2H, -CH<sub>2</sub>CH<sub>2</sub>COO-), 2.46-1.75 (m, 6H, -CH<sub>2</sub>CH<sub>2</sub>COO- and -COOCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N-), 1.59 (s, 4H, -COOCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N-).

PHDLG-BIm<sub>40</sub> was synthesized similarly using PCHDLG<sub>40</sub> as the starting material. <sup>1</sup>H NMR (D<sub>2</sub>O): δ 7.71 (s, 1H, -NCHN-), 7.52 (m, 4H, Ar-H), 4.66 (s, 1H, α-H), 4.36 (m, 2H, -COOCH<sub>2</sub>(CH<sub>2</sub>)<sub>4</sub>CH<sub>2</sub>N-), 3.89 (s, 3H, -NCH<sub>3</sub>), 3.74 (s, 2H, -COOCH<sub>2</sub>(CH<sub>2</sub>)<sub>4</sub>CH<sub>2</sub>N-), 2.32 (s, 2H, -CH<sub>2</sub>CH<sub>2</sub>COO-), 2.16-1.75 (m, 4H, -CH<sub>2</sub>CH<sub>2</sub>COO- and -COOCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N-), 1.49-0.89 (s, 6H, -COOCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N-).

PHLG-BIm<sub>28</sub> was synthesized similarly using PCHLG<sub>28</sub> as the starting material. <sup>1</sup>H NMR (TFA-d): δ 9.02 (s, 1H, -NCHN-), 7.86 (m, 4H, Ar-H), 4.86 (s, 1H, α-H), 4.36 (m, 2H, -COOCH<sub>2</sub>(CH<sub>2</sub>)<sub>4</sub>CH<sub>2</sub>N-), 4.29 (s, 2H, -COOCH<sub>2</sub>(CH<sub>2</sub>)<sub>4</sub>CH<sub>2</sub>N-), 4.24 (s, 3H, -NCH<sub>3</sub>), 2.72 (s, 2H, -CH<sub>2</sub>CH<sub>2</sub>COO-), 2.46-1.75 (m, 6H, -CH<sub>2</sub>CH<sub>2</sub>COO- and

-COOCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N-), 1.59 (s, 4H, -COOCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N-).

**Simulation Methods.** We conducted molecular dynamics simulations of PHLG-BIm with a DP of 20 following a procedure similar to that we previously detailed in Ref. (1). The non-natural peptide side chains were constructed using the Automated Topology Builder (ATB) server (<http://compbio.biosci.uq.edu.au/atb/>) (2,3,4) to define the structure, partial charges, and bonded and non-bonded interactions within the GROMOS 54A7 force field (5). The peptide backbone was initialized in a  $\alpha$ -helical conformation with the assistance of the Bax Group PDB Utility Server (<http://spin.niddk.nih.gov/bax/nmrserver/pdbutil>). An in-house code was used to graft the side chains to the backbone to synthesize the initial peptide structure. The peptide was prepared as a zwitterion, and the terminal benzimidazole groups' protonated, such that the peptide carries a net formal charge of (+20). The peptide was placed in a 9×9×9 nm cubic simulation box with periodic boundary conditions, and solvated by SPC water molecules (6) to a density of 1.0 g/cm<sup>3</sup> along with 20 Cl<sup>-</sup> counter ions such that the system carried no net charge. The size of the simulation box was specified such that with a 1.2 nm real-space cutoff each solvent molecule was able to interact with at most one periodic image of the peptide, even in its fully extended conformation.

Molecular dynamics simulations were conducted using the GROMACS 4.6 simulation



suite (7). High-energy overlaps in the initial configuration were eliminated by steepest descent energy minimization to remove forces exceeding 1000 kJ/mol.nm. Simulations were performed in the NPT ensemble at 298 K and 1 bar, employing a Nosé-Hoover thermostat (8) and Parrinello-Rahman barostat (9). Initial atom velocities were randomly assigned from a Maxwell distribution at 298 K. The equations of motion were numerically integrated using a leap-frog algorithm (10) with a 2 fs time step, and bond lengths fixed using the LINCS algorithm to improve efficiency (11). Electrostatic interactions were treated using Particle Mesh Ewald (PME) with a real-space cutoff of 1.2 nm and a 0.12 nm Fourier grid spacing (12). Lennard-Jones interactions were shifted smoothly to zero at a 1.2 nm, and Lorentz-Berthelot combining rules used to determine interaction parameters between unlike atoms (13). A 1.5 ns equilibration run was conducted, at which time the temperature, pressure, energy, and peptide radius of gyration had attained stable values. We then performed a 68 ns production run, harvesting snapshots of the system configuration for analysis every 5 ps. The peptide remained fully alpha-helical over the entire course of the simulation trajectory. We present in the Supplementary Information a movie of the simulation trajectory showing the dynamical evolution of the side chains around the  $\alpha$ -helical core (Movie S1), and a 360° rotation around a representative peptide configuration (Movie S2).

**Minimal inhibition concentration (MIC).** Gram-negative bacteria, DH5 $\alpha$  (*E. coli*),

MG1655 (*E. coli*), C101 (*Pseudomonas aeruginosa*), and Gram-positive bacteria, ATCC12608 (*S. aureus*), ATCC11778 (*Bacillus toyonensis*), and methicillin-resistant *S. aureus* (MRSA) strains, NRS382, NRS383, NRS384 were grown in LB medium at 37 °C. Clinical isolated *Helicobacter pylori* strains, B107, J291, J99, J99-AF, J99-A9 and J99-A11 were incubated in BB with 10% FBS supplemented with vancomycin (5 µg/mL) at 37 °C. Among them, J99-AF, J99-A9 and J99-A11 are drug resistant bacteria. For determination of the MIC, polypeptides were dissolved in media using serial dilutions from a stock solution. Into each well of a 96-well plate was added 200 µL of each concentration and 2 µL of bacteria ( $1 \times 10^8$  CFU (colony forming units)) in medium. The plate was incubated at 37 °C. The optical density readings of microorganism solutions were measured after 24 h incubation. The MIC was considered as the lowest concentration of peptide where no visual growth of bacteria was detected.

The stability of polypeptide was tested in the MIC assay in different environments, including salts, serum, plasma, tear fluid, and mucin.  $1 \times 10^6$  CFU/ml of *E. coli* DH5 $\alpha$  and *S. aureus* ATCC12608 were treated with peptides, while different conditions were added to LB medium, and the final concentrations of physiological conditions were as follows: 150 mM NaCl, 1 mM MgCl<sub>2</sub>, 2.5mM CaCl<sub>2</sub>, 2% human serum, 5% FBS, 10% FBS, 2% plasma, 5% plasma, 2% artificial tear (from Boss Safety Products) and 1 mg/mL mucin. After these treatments, the procedures were same as MIC assay described above.

**Killing kinetics.** The killing kinetics and killing efficiency of PHLG-BIm were measured against the microbes by counting the colony forming units of live bacteria with agar plating. The bacteria were prepared using the same procedure described in the MIC measurement. The samples were treated with PHLG-BIm<sub>40</sub> at MIC or double of the MIC and incubated at 37 °C under constant shaking (100 rpm). Samples were taken for a series of ten-fold dilutions, and plated out in LB agar plates at predetermined time intervals (1 h, 2 h, 8h and 24h). The plates were incubated over night at 37 °C and the bacteria were counted by CFU. The bacteria (DH5 $\alpha$ , ATCC12608) were also incubated with PHLG-BIm<sub>40</sub> at various concentrations without or with NaCl (150 mM), 2% human serum, 2% plasma, 2% artificial tear. The bacteria were prepared using the same procedure described in the MIC measurement. After 8 h incubation, Samples were taken for a series of ten-fold dilutions, and plated out in LB agar plates, and the bacteria were counted by CFU after overnight incubation at 37 °C.

**Hemolytic assay.** Fresh rabbit blood was obtained and subjected to 25-fold dilution with PBS buffer to reach a concentration of approximately 4% (in volume) of the blood cells. 300  $\mu$ L of PBS solution containing a polymer at various concentrations was placed in a 1.5 mL microfuge tube, followed by the addition of an equal volume (300  $\mu$ L) of red blood cell suspension. The mixture was incubated at 37 °C for 1 h to allow for the hemolysis process to take place. At the end of the incubation time, the non-hemolysed red blood cells were separated by centrifugation at 1000 rpm for 5 min. Aliquots (100  $\mu$ L) of

the supernatant were transferred to a 96-well plate, and hemoglobin release was measured by UV-absorbance at 576 nm using a microplate reader (TECAN, Switzerland). Two controls were provided in this assay: an untreated red blood cell suspension in PBS solution was used as the negative control; a solution containing red blood cells lysed with 1% Triton-X was used as the positive control. Percentage of hemolysis was calculated using the following formula: Hemolysis (%) = [(O.D. 576 nm of the treated sample-O.D. 576 nm of the negative control)/(O.D. 576 nm of positive control- O.D. 576 nm of negative control)]×100%.

**The stability of polypeptides against protease.** PHLG-BIm (1.0 mg/mL) was incubated with trypsin (1.0 mg/mL) or pronase (0.12 mg/mL) or *Pseudomonas Aeruginosa* elastase (6.25 mg/L) in Tris buffer (pH 7.4) at 37 °C. PHLG-BIm (1.0 mg/mL) was incubated with elastase from human leukocytes (25 mg/L) in sodium acetate buffer (0.05 M, pH 5.5, with 0.6 M NaCl) at 37 °C. As a positive control, LL-37 (0.1 mg/mL) was also incubated with trypsin (0.1 mg/mL) or pronase (12 mg/L) or *Pseudomonas Aeruginosa* elastase (0.6 mg/L) in Tris buffer (pH 7.4) at 37 °C. LL-37 (0.1 mg/mL) was incubated with elastase from human leukocytes (2.5 mg/L) in sodium acetate buffer at 37 °C. After 8 h of incubation, the samples were taken out for HPLC analyses. In a separate experiment, PHLG-BIm (1.0 mg/mL) was incubated with trypsin (1.0 mg/mL) or pronase (0.12 mg/mL) in Tris buffer (pH 7.4) at 37 °C for 8 h and MIC measurement was conducted.

**Flow cytometry analysis of pore-forming activities.** For membrane permeability assay,  $1 \times 10^6$  CFU MG1655 cells were combined with propidium iodide (PI) (final concentration 25  $\mu$ M), HEPES (1 mM), glucose (1 mM) and polypeptide, and incubated for 15 min at room temperature. Changes in cell-associated dye fluorescence were measured with a BD Biosciences LSR II flow cytometer, using excitation at 488 nm with an argon laser and measurement of emission through a band-pass filter at 695/40 nm for PI. A minimum of 25,000 events were detected for each sample. Calculation the geometric mean fluorescence intensity (MFI) of each population was performed using FCS Express 3.00.0311 V Lite Stand-alone software.

**Fluorescence microscopy of stained bacterial cells.** A Zeiss XBO 75 Fluorescence Microscope (Carl Zeiss) was used for fluorescence studies. A BacLight™ Kit L-7012 was used as the fluorescence dye in a mixture of propidium iodide : SYTO9 to examine bacteria in the presence of polypeptides. It is important to mention that an initial bacterial concentration of  $\sim 10^8$  cells/mL was used for microscopy for ease of visualization. The dye mixture was incubated with the bacteria at room temperature for 15 min prior to the addition of polypeptide solution. Solution of cells, dye, and polymer were allowed to stand for 30 min, and 50  $\mu$ L of the solution was placed on a slide, mounted with a coverslip, and visualized under fluorescence microscope. Bacteria were viewed under a green filter (excitation/emission, 420-480 nm/520-800 nm) or a red filter (480-550 nm/590-800 nm).

**Liposome dye leakage assay.** Calcein dye was dissolved in Tris buffer (pH = 7) to achieve a concentration of 40 mM. To a clean round-bottom flask, appropriate volumes of lipid stocks were added to make up 1 mL of CHCl<sub>3</sub> (For 3:1 POPE (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine)/POPG (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-(1'-*rac*-glycerol) (sodium salt)) vesicles, POPE (130 μL, 25 mg/mL CHCl<sub>3</sub>) and POPG (115 μL, 10 mg/mL CHCl<sub>3</sub>) were used; For DOPC (1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-phosphocholine)/POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine) vesicles, DOPC (100 μL, 25 mg/mL CHCl<sub>3</sub>) and POPC (1 mL, 10 mg/mL CHCl<sub>3</sub>) were used). The solvent was removed by a stream of nitrogen gas to obtain a thin lipid film, which was then hydrated by 1 mL of calcein solution. The mixture was left to stir for 1 h, after which it was subjected to 10 freeze–thaw cycles (using dry ice/acetone to freeze and warm water to thaw). The suspension was extruded 20 times through a polycarbonate membrane with 400 nm pore diameter. The excess dye was removed using Sephadex G-50 column as the eluent. The dye-filled vesicle fractions were diluted 200 times with Tris buffer. This suspension (90 μL) was subsequently mixed with polypeptide stock solutions (10 μL) on a 96-well black microplate (Greiner, flat bottom). Tris buffer (10 μL) and Triton-X (0.1% v/v, 10 μL) were employed as the negative and positive controls, respectively. After 30 min, the fluorescence intensity in each well was recorded using the microplate reader with excitation and emission wavelengths of 490 and 515 nm, respectively. The percentage of

leaked calcein dye in each well was determined as follows: leakage (%) =  $[(F-F_0)/(F_{TX}-F_0)] \times 100\%$ , where F is the fluorescence intensity recorded in the well, F<sub>0</sub> is the intensity in the negative control well, and F<sub>TX</sub> is the intensity in the positive control well.

**SEM analysis.** MG1655 bacterial cells grown in LB with or without polypeptides treatment were performed using a similar protocol as MIC measurements but with a 30 min incubation time. All the samples were collected into a microfuge tube and pelleted at 4000 rpm for 5 min, and then washed twice with phosphate-buffered saline. Subsequently, bacteria were fixed with paraformaldehyde solution (4%) for 1 h before proceeding, followed by washing with DI water. Dehydration was performed with a series of graded ethanol solution (10%, 25%, 50%, 75%, 95%, and 100%). The dehydrated samples were dried under vacuum overnight before being mounted on carbon tape and coated with gold-platinum for imaging using a Hitachi S-4700 High Solution SEM (Japan).

**Liposome preparation for X-ray measurements.** DOPS (1,2-dioleoyl-*sn*-glycero-3-phospho-L-serine (sodium salt)), DOPE (1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine), DOPC (1,2-dioleoyl-*sn*-glycero-3-phosphocholine), DOPG (1,2-dioleoyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)] (sodium salt)), and CL (bovine heart cardiolipin (sodium salt)), lyophilized lipids from Avanti Polar Lipids, were used

without further purification to form small unilamellar vesicles (SUVs). Individual lipid stock solutions of DOPS, DOPE, DOPC, DOPG, and CL were prepared in chloroform at a concentration of 20 mg/mL. Mixtures of these lipids were prepared at molar ratios to yield each model membrane composition. The lipid solution mixtures were placed under N<sub>2</sub> to evaporate chloroform, and were further dried by overnight desiccation under vacuum. The dried lipid mixtures were solubilized the following day in 100 mM NaCl, 10 mM HEPES at pH 7.4 to a concentration of 20 mg/mL. These aqueous lipid solutions were incubated at 37 °C overnight and then sonicated until clear. SUVs were obtained by extrusion of sonicated lipid solution through a 0.2 μm pore Nucleopore filter (Whatman).

**Small-angle X-ray scattering experiments.** Polypeptide and SUVs were mixed at specific P/L molar ratios. Samples were prepared in 100 mM NaCl, 10 mM HEPES at pH 7.4 and hermetically sealed in quartz capillaries (Hilgenberg GmbH, Mark-tubes). Small-angle X-ray scattering (SAXS) experiments were conducted at the Stanford Synchrotron Radiation Light source (SSRL, BL 4-2) using monochromatic X-rays with an energy of 9 keV. The scattered radiation was collected using a Rayonix MX225-HE detector (pixel size of 73.2 μm). No radiation damage was observed for the incident beam intensities and the exposure times used. 2D SAXS powder patterns were integrated using the Nika 1.50 package for Igor Pro 6.21 and FIT2D.

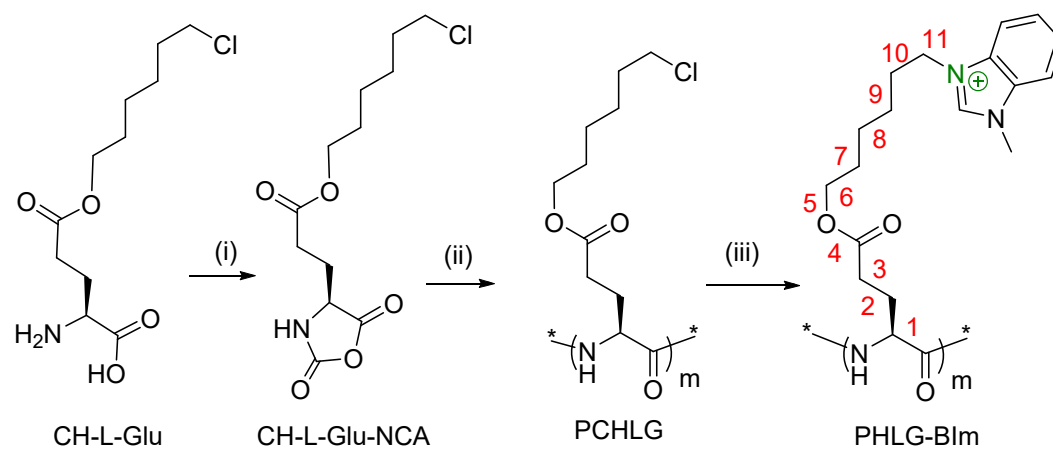
**SAXS Data Fits.** Q positions of the diffraction peaks were obtained by visual inspection



of the integrated  $I(Q)$  vs.  $Q$  SAXS data graphed in Origin Lab software. The ratios between the measured peak positions ( $Q_{(hkl)_{\text{meas}}}$ ) were compared with those of permitted reflections for different crystal phases to determine the phases present in each sample. After identifying each crystal phase, a linear regression was fit through the set of points corresponding to the reflections, with each of these points having coordinates of its  $Q$ -position,  $Q_{(hkl)_{\text{meas}}}$ , and the associated reflection in terms of Miller indices,  $h, k, l$ . For a powder-averaged cubic phase,  $Q_{(hkl)_{\text{meas}}} = (2\pi/a)\sqrt{(h^2 + k^2 + l^2)}$ . As such, with a linear regression of  $Q_{(hkl)_{\text{meas}}}$  vs.  $\sqrt{(h^2 + k^2 + l^2)}$  for cubic phases, we can calculate the cubic lattice parameter from the slope ( $m = 2\pi/a$ ).

**The intracellular uptake of rifampicin.** Bacterial strains MG1655 and C101 ( $1 \times 10^6$  CFU) were incubated with rifampicin (4.85  $\mu\text{M}$  and 38.9  $\mu\text{M}$ , respectively) without polypeptide or with PHLG-BIm<sub>40</sub> (3.3  $\mu\text{M}$ ) or with PHDLG-BIm<sub>40</sub> (3.3  $\mu\text{M}$ ). After 0.5 h or 1 h incubation, the supernatant was obtained by filtration through 0.2  $\mu\text{m}$  membrane and used for HPLC analysis.

**Scheme S1.** Synthesis of PHLG-BIm polypeptide.



(i) phosgene (ii) HDMS/DMF (iii) 1-methylbenzimidazole, NaI, DMF/MeCN

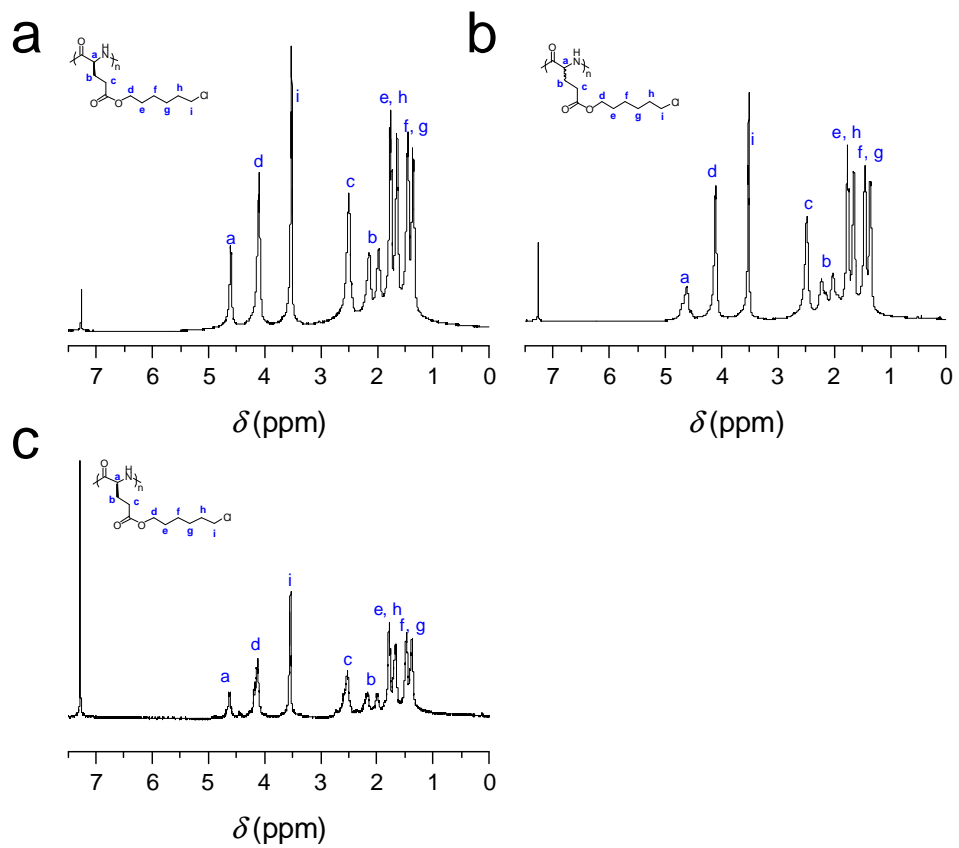
**Table S1.** Synthesis of Polypeptides with Chloroalkyl Side Chains.<sup>a</sup>

Polypeptide	Monomer	M/I	$M_n$ (kDa) <sup>b</sup>	$M_w/M_n$ <sup>b</sup>
PCHLG <sub>40</sub>	CH-L-glu-NCA	40/1	10.0	1.02
PCHDLG <sub>40</sub>	CH-DL-glu-NCA	40/1	9.7	1.10
PCHLG <sub>28</sub>	CH-L-glu-NCA	25/1	7.0	1.21

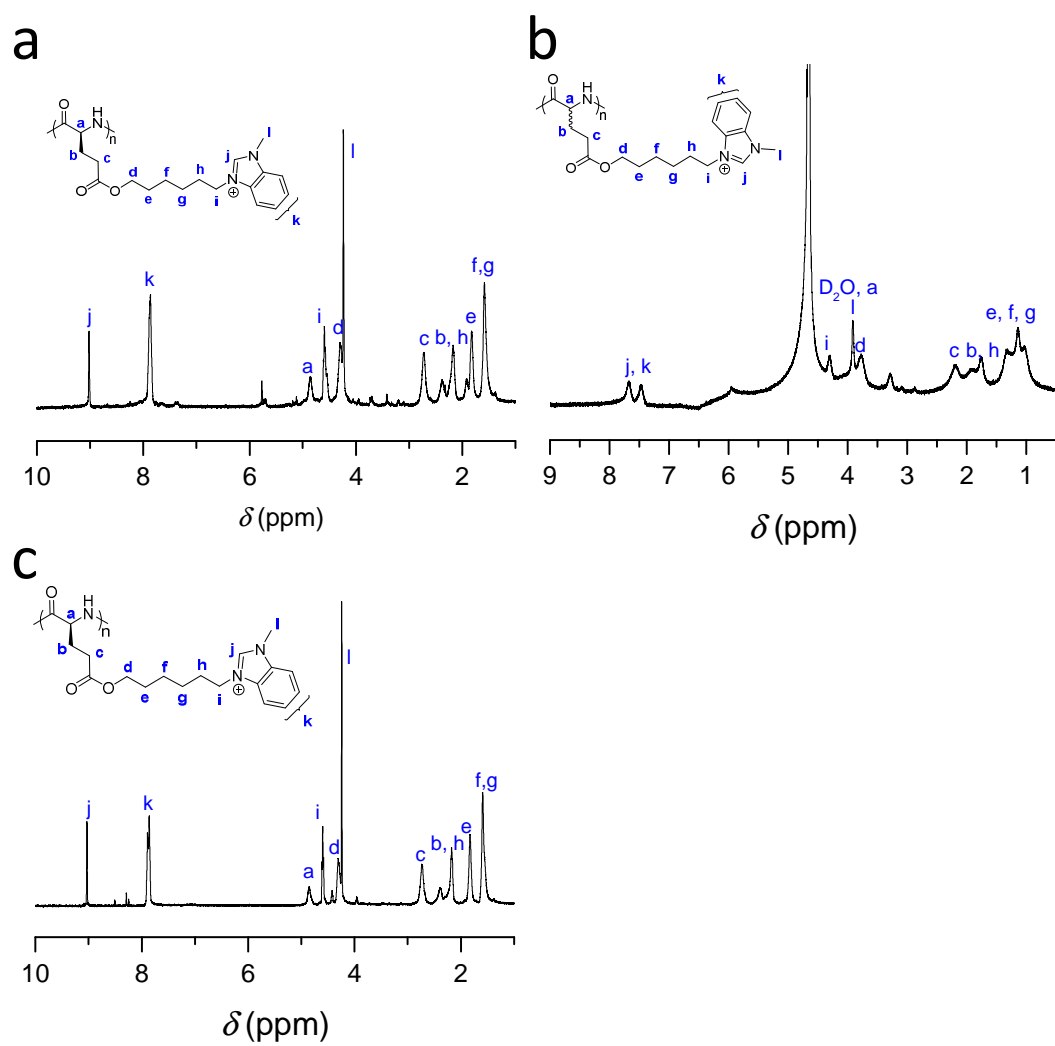
<sup>a</sup> Polymerizations were carried at room temperature for 48 h. Monomer conversions were all above 99%. <sup>b</sup> Determined by GPC.

**Table S2.** MIC values of PHLG-BIm<sub>40</sub> in the presence of salts (150 mM NaCl, 1mM MgCl<sub>2</sub>, 8  $\mu$ M CaCl<sub>2</sub>), human serum (2%), fetal bovine serum (FBS, 5% or 10%), plasma from human (2% or 5%), artificial tear (2%) or mucin (1 mg/mL) against *E. coli* DH5 $\alpha$  and *S. aureus* ATCC12608. Control represents treated with polypeptide only.

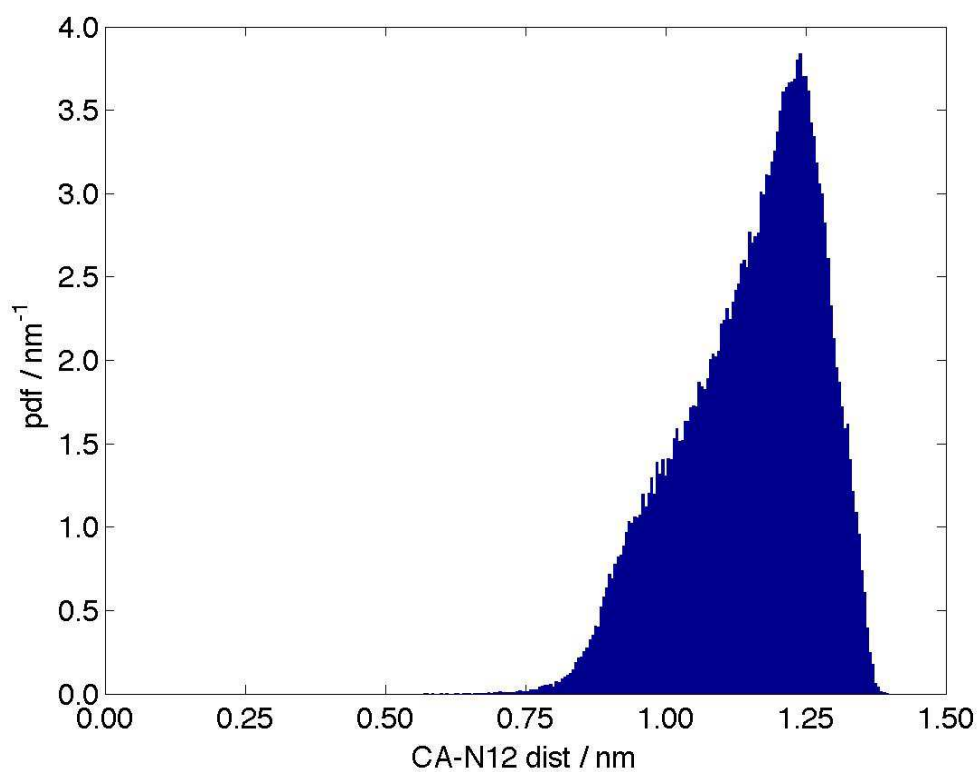
Condi on	MIC ( $\mu$ M)										
	Con trol	NaCl	MgCl <sub>2</sub>	CaCl <sub>2</sub>	2% Human Serum	5% FBS	10% FBS	2% Plasma	5% Plasma	2% Artificial tear	Mucin
DH5 $\alpha$	3.3	3.3	3.3	3.3	1.5	1.5	0.7	1.5	0.7	3.3	3.3
ATCC1 2608	13.1	13.1	13.1	6.6	6.6	6.6	3.3	6.6	3.3	6.6	13.1



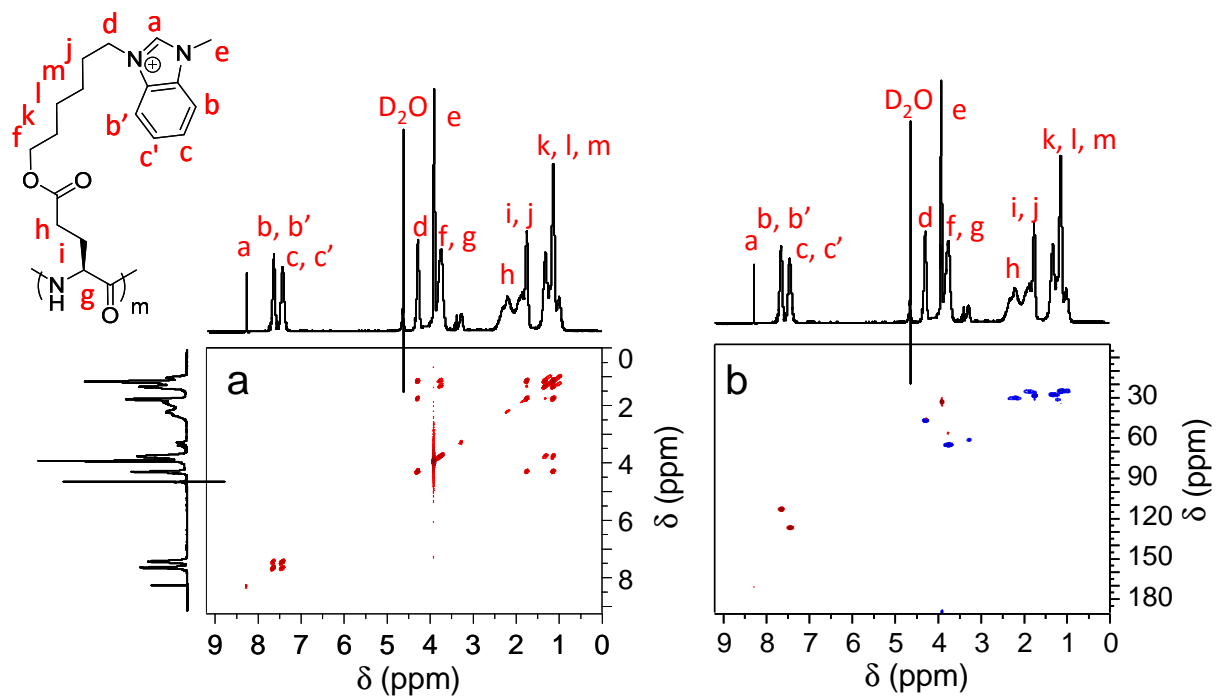
**Figure S1.** The  $^1\text{H}$  NMR spectra of PCHLG<sub>40</sub> (a), PCHDLG<sub>40</sub> (b) and PCHLG<sub>28</sub> (c) in  $\text{CDCl}_3/\text{d-TFA}$  (7/1, v/v) .



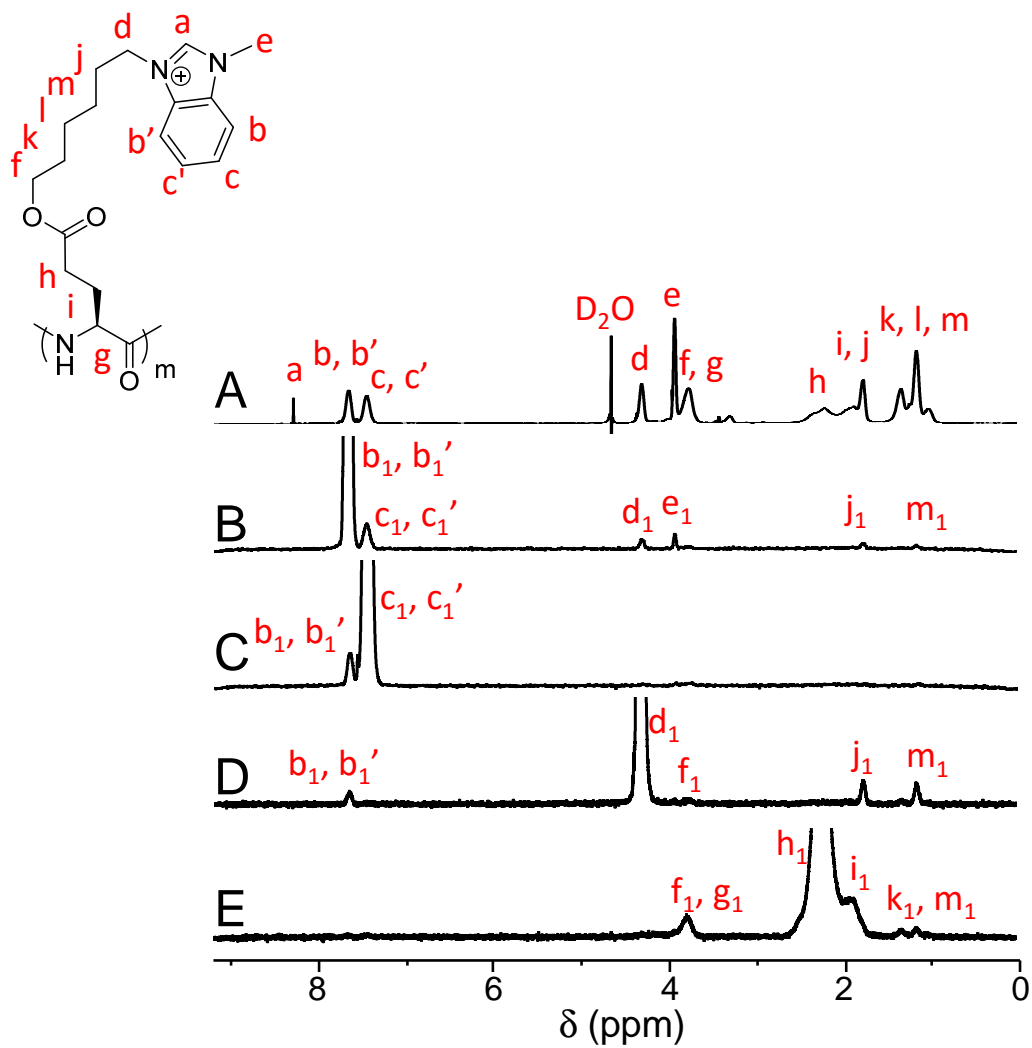
**Figure S2.** The  $^1\text{H}$  NMR spectra of PHLG-BIm<sub>40</sub> (a, TFA-d), PHDLG-BIm<sub>40</sub> (b, D<sub>2</sub>O), and PHLG-BIm<sub>28</sub> (c, TFA-d).



**Figure S3.** The probability distribution of side chain lengths measured from the  $C_{\alpha}$  to the  $N_{12}$  atom extracted from the molecular simulation trajectory and averaged over all 20 side chains with a bin resolution of  $0.005 \text{ nm}^{-1}$ . The mode of the distribution is 1.24 nm, and the mean value is 1.15 nm with a 95% confidence interval of [0.90, 1.33] nm.

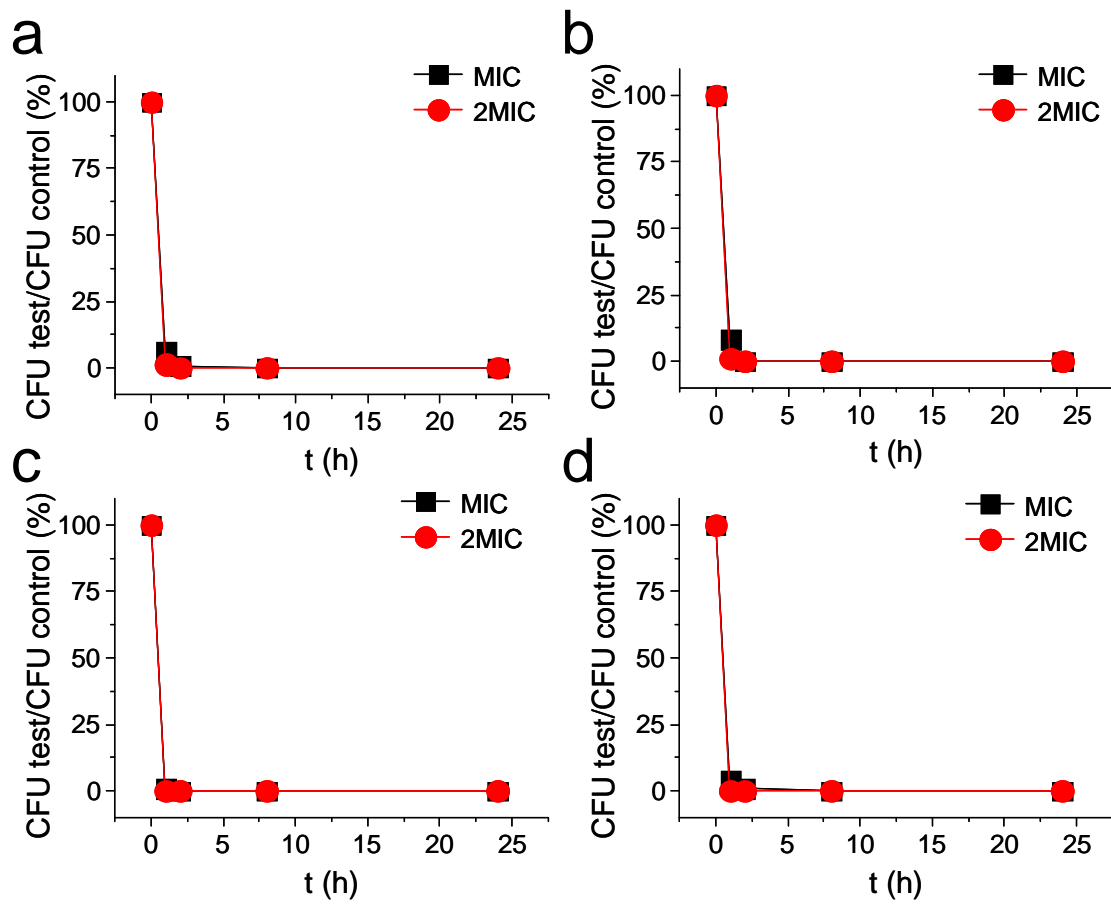


**Figure S4.** <sup>1</sup>H-<sup>1</sup>H TOCSY NMR (a, CH<sub>2</sub>, blue; CH, red) and <sup>13</sup>C-<sup>1</sup>H HSQC NMR experiments (b) for PHLG-BIm<sub>40</sub> in D<sub>2</sub>O.

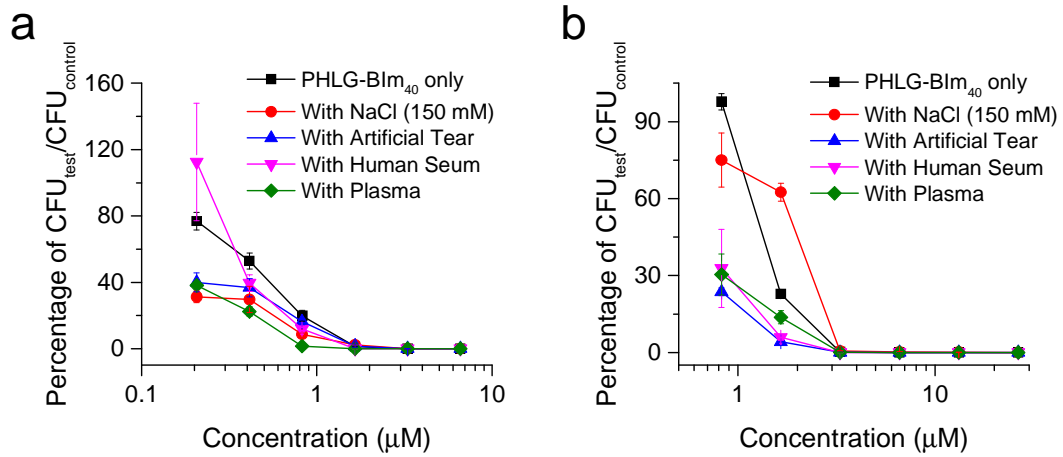


**Figure S5.** One-dimensional Proton NOE spectra of PHLG-BIm<sub>40</sub>. A) <sup>1</sup>H spectrum; B) NOESY1D from b<sub>1</sub> and b<sub>1</sub>'; C) NOESY1D from c<sub>1</sub> and c<sub>1</sub>'; D) NOESY1D from d<sub>1</sub>; E) NOESY1D from h<sub>1</sub>. A mixing time of 150 ms was used for the NOESY1D experiments.

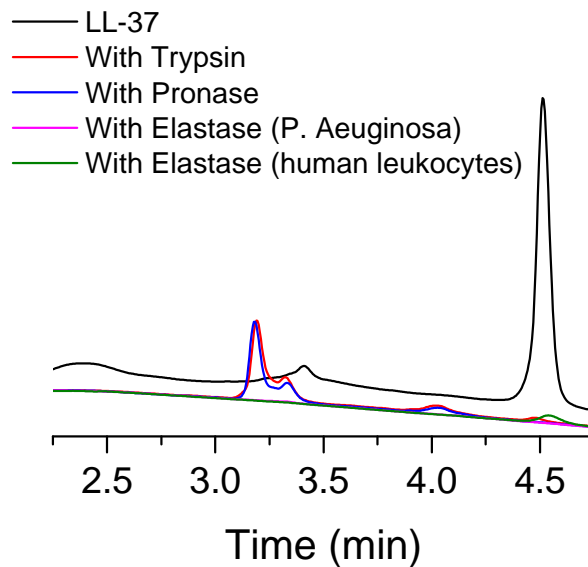




**Figure S6.** The killing kinetics of PHLG-BIm<sub>40</sub> against DH5 $\alpha$  (a), MG1655 (b), ATCC12608 (c) and ATCC11778 (d).



**Figure S7.** Percentage of CFU of DH5α (a) and ATCC12608 (b) in the samples treated with PHLG-BIm40 only, PHLG-BIm40 with NaCl (150 mM), artificial tear (2%), human serum (2%), or plasma (2%), at various concentrations for 8 h as compared to the control sample without any treatment.



**Figure S8.** The stability of LL-37 when incubated with trypsin or pronase, or elastrase from *P. aeuginosa*, or elastrase from human leukocytes for 8 h.

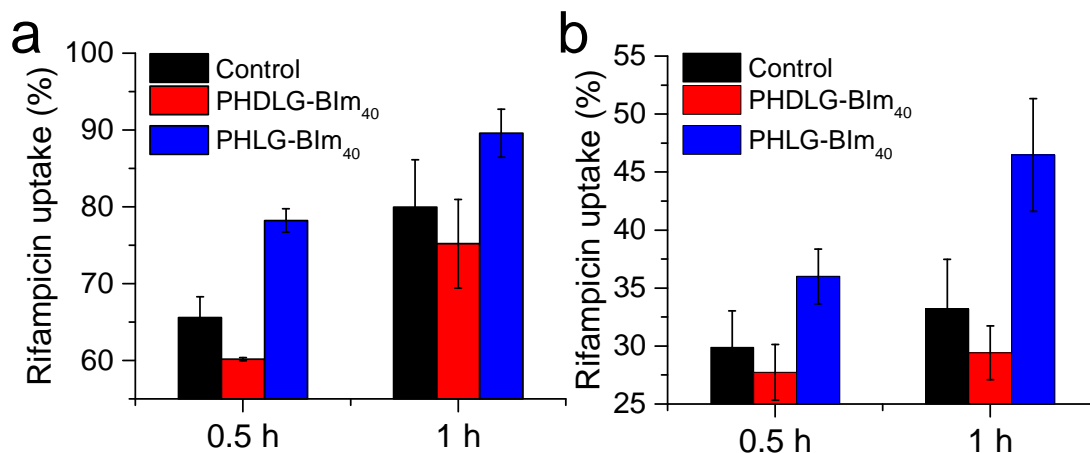
**Table S3.** The Antimicrobial Activity of Streptomycin Co-administered with PHLG-BIm or PHDLG-BIm at Various Concentrations against DH5 $\alpha$  (MIC of PHLG-BIm: 3.3  $\mu$ M), ATCC11778 (MIC PHLG-BIm: 26.1  $\mu$ M) and ATCC12608 (MIC PHLG-BIm: 26.1  $\mu$ M).

Polypeptide	MIC (DH5 $\alpha$ , $\mu$ M, $\Sigma$ FIC <sup>a</sup> )	MIC (ATCC11778, $\mu$ M, $\Sigma$ FIC <sup>a</sup> )	MIC (ATCC12608, $\mu$ M, $\Sigma$ FIC <sup>a</sup> )
Without polypeptide	6.9	6.9	6.9
With PHDLG-BIm <sub>40</sub> (1.6 $\mu$ M)	6.9 (1.5)	6.9 (1.1)	6.9 (1.1)
With PHLG-BIm <sub>40</sub> (0.8 $\mu$ M)	1.7 (0.5)	1.7 (0.3)	3.4 (0.6)
With PHLG-BIm <sub>40</sub> (1.6 $\mu$ M)	0.05 (0.5)	0.4 (0.2)	0.05 (0.1)

<sup>a</sup>  $\Sigma$ FIC = MIC<sub>A,combination</sub>/MIC<sub>A,alone</sub> + MIC<sub>B,combination</sub>/MIC<sub>B,alone</sub>. Synergy is defined as  $\Sigma$ FIC index  $\leq 0.5$ . Indifference was defined as  $\Sigma$ FIC index of  $>0.5$  but  $\leq 4$ . Antagonism was defined as  $\Sigma$ FIC index  $>4.0$ .

**Table S4.** The Antimicrobial Activity of Various Antibiotics Co-administered with PHLG-BIm (3.3  $\mu$ M) or PHDLG-BIm (3.3  $\mu$ M) against MG1655 (MIC of PHLG-BIm: 26.1  $\mu$ M) and C101 (MIC PHLG-BIm: 52.3  $\mu$ M).

Bacteria	Polypeptide	MIC (Doxycycline, $\mu$ M, $\Sigma$ FIC)	MIC (Rifampicin, $\mu$ M, $\Sigma$ FIC)	MIC (Gentamicin, $\mu$ M, $\Sigma$ FIC)
MG1655	Without polypeptide	4.5	9.7	2.1
	With PHLG-BIm <sub>40</sub>	0.6 (0.2)	0.125 (0.1)	0.5 (0.4)
	With PHDLG-BIm <sub>40</sub>	4.5 (1.1)	0.2 (1.1)	2.1 (1.1)
C101	Without polypeptide	288.0	77.8	8.4
	With PHLG-BIm <sub>40</sub>	3.6 (0.2)	2.4 (0.2)	1.0 (0.2)
	With PHDLG-BIm <sub>40</sub>	288.0 (1.1)	77.8 (1.1)	8.4 (1.1)



**Figure S9.** The intracellular uptake of rifampicin in MG1655 (a) and C101 (b) after incubated with PHDLG-BIm<sub>40</sub> (3.3  $\mu$ M) or PHLG-BIm<sub>40</sub> (3.3  $\mu$ M) for 0.5 h and 1 h.

**Movie S1.** Movie of the 68 ns production simulation with frames rendered every 150 ps. For clarity of viewing, the peptide backbones in each configuration have been mutually aligned to a single reference configuration and water molecules removed. The peptide backbone is colored in red and the side chains in grey. This movie was produced using VMD (14).

**Movie S2.** A 360° rotation around a representative peptide configuration. For clarity of viewing, the water molecules have been removed and the backbone and side chains colored in red and grey, respectively. This movie was produced using VMD (14).

**Movie S3.** Fly-around of the 3D probability distribution of the side chain N12 atoms

around the  $\alpha$ -helical backbone. Histograms were compiled over the simulation trajectory using a grid comprising cubic cells of size  $0.1 \times 0.1 \times 0.1$  nm, and the probability density function computed by normalizing by the total count and the cell volume. The value of the probability density in any one cell ranged from  $0-3.7$  nm<sup>-3</sup>. Four contours in the probability density are plotted at 0.05, 0.1, 0.2, and 0.3 nm<sup>-3</sup>. Since the positive charge in the cationic side chains resides primarily in the termini, this distribution may be considered a proxy for that of the positive charge. This movie was produced using MATLAB (15).

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