Supporting information for

Selective Killing of *Helicobacter pylori* with pH-Responsive Helix-Coil Conformation Transitionable Antimicrobial Polypeptides

Menghua Xiong\(^1,^\$\), Yan Bao\(^3,^\$\), Xin Xu\(^2,^\$\), Hua Wang\(^1\), Zhiyuan Han\(^1\), Zhiyu Wang\(^8\), Yeqing Liu\(^10\), Songyin Huang\(^10\), Ziyuan Song\(^1\), Jinjing Chen\(^3\), Richard M. Peek, Jr\(^4\), Lichen Yin\(^2,*\), Lin-Feng Chen\(^3,*\), Jianjun Cheng\(^1,^\,^2,^\,^5-^9,*\)

\(^1\)Department of Materials Science and Engineering, University of Illinois at Urbana-Champaign, 1304 West Green Street, Urbana, IL, 61801, USA.
\(^2\)Jiangsu Key Laboratory for Carbon-Based Functional Materials & Devices, Institute of Functional Nano & Soft Materials (FUNSOM), Collaborative Innovation Center of Suzhou Nano Science & Technology, Soochow University, Jiangsu, China.
\(^3\)Department of Biochemistry, University of Illinois at Urbana-Champaign, 600 S Mathews Ave, Urbana, IL 61801, USA.
\(^4\)Division of Gastroenterology, Department of Medicine and Cancer Biology, Vanderbilt University School of Medicine, Nashville, TN 37232, USA.
\(^5\)Department of Bioengineering, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA.
\(^6\)Beckman Institute for Advanced Science and Technology, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA.
\(^7\)Department of Chemistry, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA.
\(^8\)Frederick Seitz Materials Research Laboratory, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA.
\(^9\)Carl R. Woese Institute for Genomic Biology, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA.
\(^10\)Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangdong, China.
**Experimental procedures:**

**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 μm) was purchased from SiliCycle Inc. (Quebec City, Quebec, Canada) and heated to 150 °C for 48 h before use. L-tert-butyl-Glu and DL-tert-butyl-Glu were purchased from Chem-impex International, INC. Spectra/Por® dialysis tubing with a molecular weight cut-off (MWCO) of 1 kDa was purchased from Spectrum Laboratories (Rancho Dominguez, CA, USA). DH5α, MG1655 (*Escherichia coli*) were grown in luria broth (LB) medium at 37 °C. *Helicobacter pylori* strain SS1, and clinical isolated strains, J68, J104, J166, J99, J99A-9, J99A-7, J99A-11, J99C-8 and J99D-1, supplied by Dr. Chen and Dr. Peek, were incubated in brucella broth (BB) with 10% fetal bovine serum (FBS) supplemented with vancomycin (5 μg/mL) in the incubation with the supplement of 10% carbon oxide at 37 °C. Among them, J99A-7, J99A-11, J99C-8 and J99D-1 are drug resistant bacteria. All lipids were obtained from Avanti Polar Lipids, Inc. BacLight™ Kit L-7012 was purchased from Thermo Fisher Scientific Inc.

**Instruments.** ¹H NMR spectra were recorded on a Varian U500 MHz or a VXR-500 MHz spectrometer. Chemical shifts were reported in ppm and referenced to the solvent proton impurities. Gel permeation chromatography (GPC) experiments were performed
on a system 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 µm, 300 × 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 the determination of the absolute molecular weights (MWs). The MWs of polymers were determined based on the dn/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 polymer samples were prepared at concentrations of 4.4 µM. The solution was placed in a quartz cell with a path length of 0.10 cm. The zeta-potential of polypeptides was evaluated by Malvern Zetasizer. Infrared spectra were recorded on a Perkin Elmer 100 serial FTIR spectrophotometer calibrated with polystyrene film.

Lyophilization was performed on a FreeZonelyophilizer (Labconco, Kansas City, MO, USA). UV light was generated from an OmiCure S1000 UV lamp (EXFO, Mississauga, Canada).

**Animals.** Female C57BL/6J mice were purchased from The Jackson Labs (Bai Harbor, ME, USA). Feed and water were available ad libitum. Artificial light was provided in a
12 h/12 h cycle. The animal protocol was reviewed and approved by the Illinois Institutional Animal Care and Use Committee (IACUC) of University of Illinois at Urbana–Champaign.

**Synthesis of L-tert-butyl-Glu-NCA (tBLG-NCA).** A round-bottomed flask (100 mL) was charged with L-tert-butyl-Glu (3 g, 14.8 mmol) and dried under vacuum for 2 h. Anhydrous tetrahydrofuran (THF, 30 mL) and phosgene (15wt% in toluene, 12.6 mL, 29.6 mmol) 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 50 % hexanes) as the eluent (2.1 g, yield: 63 %). ¹H NMR (CDCl₃, ppm): δ 6.70 (s, 1H, -NH), 4.39 (t, 1H, -CHNH), 2.48 (t, 2H, -CH₂CH₂COO-), 2.25-2.06 (m, 2H, -CH₂CH₂COO-), 1.46 (s, 9H, C(CH₃)₃). ¹³C NMR(CDCl₃, ppm): δ 172.3, 169.8, 152.6, 82.2, 57.5, 31.5, 28.3, 27.2.

tBDLG-NCA was synthesized similarly using tBDLG as the starting material (Yield: 71 %). ¹H NMR (CDCl₃, ppm): δ 6.82 (s, 1H, -NH), 4.37 (t, 1H, -CHNH), 2.47 (t, 2H, -CH₂CH₂COO-), 2.26-2.06 (m, 2H, -CH₂CH₂COO-), 1.46 (s, 9H, C(CH₃)₃). ¹³C NMR (CDCl₃, ppm): δ 172.4, 169.8, 151.8, 82.3, 57.6, 31.6, 28.3, 27.3.

**Synthesis of random copolymers poly(γ-6-chlorohexyl-L-glutamate)-r-poly(tert-butyl-L-Glu) (PCHLG-r-PtBLG).** L-γ-(6-chlorohexyl)-Glu-NCA (CH-L-Glu-NCA) were synthesized according to our previous work (1). In the glovebox, CH-L-glu-NCA, L-tert-butyl-Glu and HMDS was mixed in various feeding molar ratios (20:20:1) and dissolved in DMF (1.5 mL). The mixture was stirred at room temperature for 48 h. The
polymers were then precipitated in cold ether/hexane (v/v=1:1) and dried under vacuum at 40 °C for 8 h. Poly(γ-6-chlorohexyl-DL-glutamate)-r-poly(DL-tert-butyl-Glu) (PCHDLG-r-PtBDLG) was synthesized by with the same method using CH-DL-glutamic acid-NCA and DL-tert-butyl-Glu as the monomers with a feeding molar ratio at 20:20 (M/I=40).

**Synthesis of antibacterial polypeptides.** PL1 was synthesized according to our previous study (1). PL2, and PDL2 were synthesized using PCHLG_{20-r-PtBLG_{18}} and PCHDLG_{25-r-PtBDLG_{20}}, respectively, to react with N-methyldihexylamine. The tert-butyl groups were removed by trifluoroacetic acid (TFA). Briefly, the polypeptides were dissolved in DMF (2 mL), NaI (3 equiv of chloro groups) was dissolved in acetonitrile (2 mL). The mixture was transferred to a 25 mL Schlenk tube into which N-methyldihexylamine (3 equiv of chloro groups) was added. 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 stirred at room temperature for 3 h to promote ion exchange. PHLG-MHH_{20-r-PtBLG_{20}} and PHDLG-MHH_{25-r-PtBDLG_{20}} were then obtained after purification by dialysis (MWCO = 1 kDa) against distilled water for 1 days and lyophilization. The polypeptides were then dissolved in TFA/methylene (1:3, v/v) and stirred for 3 h under room temperature. The PL2 and PDL2 were then obtained after purification by dialysis (MWCO = 1 kDa) against distilled water for 1 days and lyophilization.

Cy5 labeled PL2 and PDL2 were synthesized as Scheme S3. Briefly, PCHLG_{20-r-PtBLG_{20}} and PCHDLG_{25-r-PtBDLG_{20}} were first react with 3-azido-N,N-
dimethylpropan-1-amine (0.2 equiv of chloro groups) and NaI (3 equiv of chloro groups) for one day, and then N-methylidihexylamine (3 equiv of chloro groups) were added. The mixture were stirred for another two days under 80 °C. The polypeptides were obtained after purification by dialysis (MWCO = 1 kDa) against distilled water for 1 days and lyophilization, followed by de-esterification using TFA. Cy5 labeled polypeptides were obtained by incubation with DBCO-cy5 (0.5 equiv of azido groups) in dimethyl sulfoxide/H2O (v/v=1:1). The conversion of DBCO-cy5 were complete after 24 h incubation, confirmed by HPLC (Fig. S5).

**Minimal inhibition concentration (MIC).** Gram-negative bacteria, DH5α and MG1655 (*E. coli*) were grown in LB medium at 37 °C. 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 × 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.

**H. pylori killing kinetics at various pHs.** The killing kinetics of polypeptides were measured against *H. pylori* by counting the colony forming units (CFU) of alive bacteria with agar plating. The polypeptides at various concentrations were dissolved in the Tris-HCl buffer at various pHs (pH 7.4, 4.0, 3.0). *H. pylori* were then collected and dispersed in the BB medium supplied with fresh urea (10 mM) and 10% FBS at different pHs (pH 7.4, 4.0, 3.0). The optical density at 600 nm of the bacteria were fixed
at 0.1. The bacteria were then incubated with the polypeptides solution at corresponding pH in the incubator supplied with 10% CO₂ at 37 °C. Samples were taken for a series of ten-fold dilutions, and plated out in BB agar plates with 10% FBS and vancomycin (pH 7.4) after 1 h incubation. The plates were incubated for 72 h in the incubator and the bacteria were counted for CFU. Bacteria incubated with Tris-HCl buffer only at corresponding pH were served as 100% survival. Survival rate (%) = CFU of bacteria treated with polypeptides/CFU of bacteria treated with buffer × 100%.

The killing kinetics of antibiotics (OAC, omeprazole, amoxicillin, clarithromycin) against H. pylori were determined with a similar procedure. The antibiotics at various concentrations were dissolved in the Tris-HCl buffer at pH 7.4, or 3.0, and were then incubated with H. pylori in BB medium supplied with fresh urea (10 mM) and 10% FBS at corresponding pH in the incubator. After 1 h incubation, the bacteria were collected by centrifuge at 10,000 rcf for 5 min and washed with fresh BB medium. Samples were then taken for a series of ten-fold dilutions, and plated out in BB agar plates with 10% FBS and vancomycin. The plates were incubated for 72 h in the incubator and the bacteria were counted for CFU.

**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 μL of PBS solution containing a polypeptide at various concentrations was placed in a 1.5 mL microfuge tube, followed by the addition of an equal volume (300 μL) 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 μL) 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 pepsin. Cy5 labeled PL2 (1.0 mg/mL) was incubated with pepsin (1.0 mg/mL) in Tris buffer (pH 4.0) at 37 °C. After 24 h of incubation, the samples were taken out for HPLC analyses.

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: SYTO9 to examine bacteria in the presence of polypeptides. It is important to mention that an initial bacterial concentration of ~10^8 cells/mL was used for microscopy for ease of visualization. SYTO9 and cy5 labeled PL2 (2.2 μM) were incubated with SS1 under pH 7.4 and pH 4.0 with the presence of urea (10 mM). Solution of cells, dye, and polymer were allowed to stand for 30 min, and 50 μL of the solution was placed on a slide, mounted with a coverslip, and visualized under fluorescence microscope.

Liposome dye leakage assay. ANTS (12.5 mM) and DPX (45 mM) were dissolved in
Tris buffer (pH = 7.4). To a clean round-bottom flask, appropriate volumes of lipid stocks were added to make up 1 mL of CHCl₃ (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 CHCl₃) and POPG (115 µL, 10 mg/mL CHCl₃) 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 ANTS/DPX solution. The mixture was left to hydrate 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) at various pH (pH 7.4 or 4.0) on a 96-well black microplate (Greiner, flat bottom). Tris buffer (10 µL, pH 7.4 or 4.0) and Triton-X (0.1% v/v, 10 µL, pH 7.4 or 4.0) were employed as the negative and positive controls at corresponding pH, respectively. After 30 min, the fluorescence intensity in each well was recorded using the microplate reader with excitation and emission wavelengths of 380 and 520 nm, respectively. The percentage of leaked calcein dye in each well was determined as follows: leakage (%) = [(F−F₀)/(F_TX−F₀)]×100%, where F is the fluorescence intensity recorded in the well, F₀ is the intensity in the negative control well, and F_TX is the intensity in the positive control well.

SEM analysis. SS1 bacterial cells grown in BB with the supplement of 10% FBS under
pH 7.4 or 4.0 with or without polypeptides (2.2 μM) treatment were performed using a similar protocol as killing kinetics 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).

**In vivo and ex vivo biodistribution of Cy5-PL2.** Cy5-PL2 (2.6 μmol/kg), Cy5-PDL2 (2.6 μmol/kg) and PBS were administrated by oral gavage. Mice were placed on the sample stage equipped with anesthesia input and output ports, and imaged by the Bruker Xtreme In-Vivo Fluorescence Imaging System at 1 h, 2 h, 4 h, 7 h, and 24 h post injection (p.i.). The excitation filter was set at 630 nm and the emission filter was set at 700 nm. Collected images were analyzed by the Bruker molecular imaging software. Stomach, intestines, liver, lung, kidneys, heart, and spleen were harvested at 4 h or 24 h p.i. of Cy5-PL2 and imaged ex vivo using the Bruker Xtreme In-Vivo Imaging System. Ex vivo images were quantified by measuring FI at selected region of interest (ROI). All values were expressed as means ± standard deviation (n = 3). After ex vivo imaging, stomachs were bisected. Half of the stomach was directly frozen in O.C.T. compound and sectioned on a cryostat (Leica CM3050S) with a thickness of 6 μm. Hoester solution (2 μg/mL) was added to stain cell nucleus. After multiple washing steps,
coverslips were mounted onto the microscope slides with the addition of ProLong Gold antifade reagent, and the prepared samples were stored in the dark for confocal imaging. The other half of the stomach were placed into separate glass tubes containing 2 mL of lysis buffer (1% SDS, 100 mM Tris.HCl, pH 7.4, 1 tablet of EDTA-free protease inhibitor), homogenized, and incubated at 4 °C for 2 h. The lysates were then centrifuged at 3000 rcf for 10 min to remove the insoluble cell debris, and measured on a fluorescence spectrometer to determine the amount of Cy5 retained in the tissues. The amount of Cy5 was calculated based on the standard curve of Cy5 FI and normalized to percentage of injected dose (% I.D.) per gram tissue.

**Anti-*H. pylori* efficacy in vivo.** Each C57BL/6J mouse received 0.2 mL *H. pylori* SS1 (OD$_{600}$=2) in BHI broth administered intragastrically through oral gavage every other day for four times (on days 1, 3, 5 and 7, respectively), and the infection was allowed to develop for 2 wk. The mice were randomly assigned to four treatment groups (n ≥ 6) to receive PL2, PDL2, triple therapy (omeprazole, amoxicillin, clarithromycin), or PBS. For triple therapy, mice were first administered omeprazole (a proton pump inhibitor) through oral gavage at a dose of 400 μmol/kg, followed by a lag time of 30 min before administration of amoxicillin (68.0 μmol/kg) and clarithromycin (19.1 μmol/kg). PL2 and PDL2 in 5% (vol/vol) DMSO (both at 2.6 μmol/kg) were administered through oral gavage once daily for a consecutive 3 d. Water solution with 5% DMSO was served as negative control group. Forty-eight hours after the last administration, mice were killed and the stomach was removed from the abdominal cavity. The stomach was cut along the greater curvature, and the gastric content was removed and rinsed with PBS. The
stomach sections were used for assessment of bacterial colonization. For bacterial colonization, a gastric tissue section was suspended in 1 mL PBS and homogenized for *H. pylori* recovery. The homogenate was serially diluted and spotted onto Columbia agar plate containing vancomycin (20 μg/mL), amphotericin (2 μg/mL), bacitracin (30 μg/mL) and nalidixic acid (10 μg/mL). The plates were then incubated at 37 °C under microaerobic conditions for 5 d, and bacterial colonies were enumerated and adjusted for dilutions.

**Toxicity of PL2 in vivo.** Mice were given a daily gavage of PL2 (2.6 μmol/kg), 5% DMSO, OAC and physiological saline for 3 consecutive days. Mice were sacrificed on day 5, and the blood samples were collected for the analysis of alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, urea nitrogen, sodium ion and potassium ion levels. The colonic segment, stomach, liver, small intestine, kidney and feces of each mouse was gathered and homogenized with cold PBS (pH 7.4). The stomachs were used to determine the expression of caspase 3 and caspase 8 using Caspase 3 Assay Kit (Sigma) and Caspase 8/FLICE Colorimetric Kit (Life technology), respectively. The colonic segment and feces were used to extract bacterial DNA. The stomach, small intestine, liver, and kidney were kept in formalin for H&E staining. The stomachs were also kept in formalin for terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay (S7100 | ApopTag® Peroxidase In Situ Apoptosis Detection Kit). The percentage of TUNEL positive cells in the stomach after control, OAC, PL2 treatments were counted from 10 different images (mean ± SD, n ≥ 5). The inflammation and injury of stomach were scored by Dr. Liu in a blind manner based
on the H&E staining. Inflammation was graded on a 0–3 ordinal scale based on the Sydney System as follows: chronic inflammation (mononuclear cell infiltration independent of lymphoid follicles); grade 0, no inflammation; grade 1, mild inflammation (slight increase in mononuclear cells); grade 2, moderate inflammation (dense but focal mononuclear inflammatory cells); and grade 3, severe inflammation (dense and diffuse mononuclear inflammatory cells). The appearance of the mucosa was graded as follows: 0, normal; 1, spotty changes in cellular staining characteristics of some surface epithelial cells in an otherwise normal mucosa (mild injury); 2, more generalized changes and/or disruption of the surface epithelium in several areas (moderate injury); 3, extensive mucosal destruction (severe injury).

The killing effect of PL2 against commensal bacteria was determined by measuring the bacterial load in the feces and ileal contents of mice after a daily gavage of PL2 (2.6 μmol/kg in 5% DMSO), 5% DMSO and OAC for 3 consecutive days. The bacterial load was determined by quantitative real-time PCR using a protocol modified from ref. (2, 3). Fecal DNA was extracted as for sequencing, and bacterial load was quantified against a standard curve derived from apCR4TOPO-TA vector containing a nearly full-length copy of the 16S rRNA gene from a member of Porphyromonadaceae. Bacterial DNA was amplified with universal primers 8F and 338R using the iQ SYBR greensupermix (Bio-RadLifeScience) and the StepOne Plus system (AppliedBiosystems). The results were normalized to ileal content/fecal weight.

**Statistical analysis.** The statistical analysis was performed by one-way analysis of variance (ANOVA) with post hoc Fisher’s Least Significant Difference (LSD) test
(OriginPro 8.5) or Student’s t-test (two-tailed) comparisons at 95% confidence interval. The results were deemed significant at \(*P \leq 0.05.*\)
Table S1. The characterization of random polypeptides PCHLG$_{20}$-$r$-PtBLG$_{18}$ and PCHDLG$_{25}$-$r$-PtBDLG$_{20}$.

<table>
<thead>
<tr>
<th>Polypeptide</th>
<th>$M_n$ (kDa)</th>
<th>$M_w/M_n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCHLG$<em>{20}$-$r$-PtBLG$</em>{18}$</td>
<td>9.4</td>
<td>1.10</td>
</tr>
<tr>
<td>PCHDLG$<em>{25}$-$r$-PtBDLG$</em>{20}$</td>
<td>12.7</td>
<td>1.28</td>
</tr>
</tbody>
</table>

Table S2. The toxicity of PL2 after oral gavage.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>Creatinine (µM)</th>
<th>Urea nitrogen (mM)</th>
<th>Potassium ion (mM)</th>
<th>Sodium ion (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS</td>
<td>32.6±4.5</td>
<td>132.3±23.3</td>
<td>9.7±1.2</td>
<td>8.2±0.4</td>
<td>8.4±0.4</td>
<td>150.2±0.7</td>
</tr>
<tr>
<td>5% DMSO</td>
<td>36.0±12.7</td>
<td>123.0±30.6</td>
<td>9.7±2.0</td>
<td>8.6±1.7</td>
<td>8.6±1.1</td>
<td>151.7±2.2</td>
</tr>
<tr>
<td></td>
<td>(P=0.3)</td>
<td>(P=0.2)</td>
<td>(P=0.5)</td>
<td>(P=0.4)</td>
<td>(P=0.4)</td>
<td>(P=0.2)</td>
</tr>
<tr>
<td>OAC</td>
<td>31.6±2.9</td>
<td>83.6±4.5</td>
<td>9±1.4</td>
<td>7.6±0.3</td>
<td>7.2±0.2</td>
<td>145.5±2.6</td>
</tr>
<tr>
<td></td>
<td>(P=0.4)</td>
<td>(P=0.02)</td>
<td>(P=0.3)</td>
<td>(P=0.09)</td>
<td>(P=0.01)</td>
<td>(P=0.04)</td>
</tr>
<tr>
<td>PL2</td>
<td>37.3±0.5</td>
<td>120.3±17.9</td>
<td>8.7±0.9</td>
<td>6.8±0.6</td>
<td>8.1±0.2</td>
<td>149.4±1.6</td>
</tr>
<tr>
<td></td>
<td>(P=0.1)</td>
<td>(P=0.1)</td>
<td>(P=0.03)</td>
<td>(P=0.05)</td>
<td>(P=0.2)</td>
<td></td>
</tr>
</tbody>
</table>

*Effect of PL2 (2.6 μmol/kg) on liver and kidney functions as well as balance of electrolytes in the blood. Physiological saline (NS), 5% DMSO and OAC were served as control groups. Data are presented as mean ± SD (n =3) and analyzed by student t test as compare with NS. $P>0.05$ means statistical insignificance, and $P\leq0.05$ means statistical significance.

Scheme S1. Synthetic route of L-tert-butyl-Glu-NCA.
**Scheme S2.** Synthetic route of antimicrobial polypeptides PL2, PL1 and PDL2.

**Scheme S3.** Synthetic route of cy5 labeled PL2.
Figure S1. The $^1$H NMR spectra of PCHLG$_{20}$-$r$-PtBLG$_{18}$ (A) in CDCl$_3$ and d-TFA (v/v 9:1), PHLG-MHH$_{20}$-$r$-PtBLG$_{18}$ (B), PL2 (C) and PL1 (D) in d-TFA (ppm).
Figure S2. The $^1$H NMR spectra of PCHDLG$_{25}$-r-PtBDLG$_{20}$ (A), PHDLG-MHH$_{25}$-r-PtBDLG$_{20}$ (B), and PDL2 (C) in d-DMSO (ppm).
Figure S3. The zeta potential of PL2 and PDL2 at different pH value adjusted from 8.0 to 3.0 (by 0.1 M HCl, A) and from 3.0 to 8.0 (by 0.1 M NaOH, B).

Figure S4. (A) CD spectra of PL1 and PDL2 under pH 7.4 or 3.0. The survival rate of H. pylori SS1 after incubation with PDL2 (B) and PL1 (C) under pH 7.4 or 3.0 for 1 h. (D) Extent of ANTS/DPX efflux in negatively charged liposome after treatment with PL1 and PDL2 at various concentrations under pH 7.4 or pH 4.0. (E) SEM images of SS1 after treatment with PL1 or PDL2 at pH 7.4 or pH 4.0. SS1 bacterial cells were incubated with or without polypeptides (2.2 μM) for 30 min under pH 7.4 or 4.0. Bar =
0.5 μm.

Figure S5. (A) The survival rate of *H. pylori* after incubation with amoxicillin (273.7 μM) and clarithromycin (133.7 μM) under pH 7.4 or 3.0 for 1 h. (B) The antibacterial activity of triple therapy OAC (a combination of omeprazole, amoxicillin and clarithromycin) against SS1 under pH 7.4 or 3.0. Concentrations of omeprazole, amoxicillin, and clarithromycin in OAC1 are 20.0 μM, 6.8 μM, and 1.9 μM, respectively; in OAC2 are 40.0 μM, 13.6 μM, and 3.8 μM, respectively; in OAC3 are 80.0 μM, 27.2 μM, and 7.6 μM, respectively. (C) The antibacterial activity of omeprazole (O) and a combination of amoxicillin and clarithromycin (AC) against SS1 at pH 3.0. O1, O2, O3 represents the concentration of omeprazole at 20.0 μM, 40.0 μM,
and 80.0 µM, respectively. AC1, AC2, AC3 represents the concentration of amoxicillin and clarithromycin at 6.8 µM and 1.9 µM, 13.6 µM and 3.8 µM, 27.2 µM and 7.6 µM, respectively. The antibiotics were incubated with *H. pylori* strains for 1 h. The antibacterial activity of amoxicillin (D) and clarithromycin (E) against clinical isolated *H. pylori* strains, J99, J99A-7, J99A-9, J99A-11, J99C-8, J99D-1. The bacteria were incubated with antibiotics under pH 7.4 in the 10% CO₂ incubator (37 °C), and the optical density at 600 nm (OD₆₀₀) of bacteria were analyzed to determine the growth of bacteria. The result indicated that J99A-7, J99A-11, J99C-8 and J99D-1 are resistant to clarithromycin. (F) The antibacterial activity of OAC against J99D-1 under pH 7.4 or 3.0. (G) The antibacterial activity of omeprazole (O) and a combination of amoxicillin and clarithromycin (AC) against J99D-1 under pH 3.0.

**Figure S6.** Fluorescent imaging of *H. pylori* SS1 after incubation with Cy5-PL2 at pH 7.4 or 4.0 for 1 h. The green fluorescence represents SYTO9, which can stain the live bacteria; and the red fluorescence represents Cy5-labeling polypeptide. At pH 7.4,
almost no Cy5 fluorescent signal was detected in the bacteria, while strong Cy5 fluorescent signal was detected in the bacteria under pH 4.0. The result indicated that more Cy5-PL2 bound to SS1 bacterial cells at pH 4.0 than at pH 7.4, which was attributed to the formation of catatonically helical structure at acidic pH that promoted binding of polypeptide molecules into phospholipid bilayers.

Figure S7. (A) HPLC analysis of Cy5-PL2 and PDL2-cy5. (B) The stability of Cy5-PL2 against pepsin after 24 h incubation.
Figure S8. Toxicity of PL2 towards mouse stomach after gavage. (A) Representative images of H&E stained stomach from mice receiving control, OAC and PL2 treatments (n≥5, scale bar = 200 µm). The inflammation score (B) and injury score (C) of stomach according to the images of H&E staining. (D) Representative TUNEL straining images of mouse stomach from mice receiving control, OAC and PL2 treatments (n≥5, scale bar = 200 µm). (E) The percentage of positive cells as calculated from TUNEL staining images. At least 10 different images of 200× area were randomly selected for each tissue to count the apoptotic cell percentage (mean ± SD, n ≥ 5). The relative activity of caspase 3 (F) and caspase 8 (G) of the stomach after control, OAC, and PL2 treatments.
The average activity of caspase 3/8 of control mice was set as 100%. Inflammation was graded on a 0–3 ordinal scale based on the Sydney System as follows: chronic inflammation (mononuclear cell infiltration independent of lymphoid follicles); grade 0, no inflammation; grade 1, mild inflammation (slight increase in mononuclear cells); grade 2, moderate inflammation (dense but focal mononuclear inflammatory cells); and grade 3, severe inflammation (dense and diffuse mononuclear inflammatory cells). The appearance of the mucosa was graded as follows: 0, normal; 1, spotty changes in cellular staining characteristics of some surface epithelial cells in an otherwise normal mucosa (mild injury); 2, more generalized changes and/or disruption of the surface epithelium in several areas (moderate injury); 3, extensive mucosal destruction (severe injury). All of the data are represented as average ± SD and analyzed by student's t-test (* *P* ≤ 0.05, **P** ≤ 0.01). “ns” represents no significant difference (*P* > 0.05).

**Figure S9.** The H&E-stained intestines, liver, and kidney from mice receiving control, OAC and PL2 (scale bar = 200 μm).
References

