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Supplementary Information

Star-Shaped Polypeptides Exhibit Potent Antibacterial Activities

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Experimental Section

Multiphoton imaging of *EHEC* treated with 3-armed PLL_{20}-*g*-$Indo_{0.2}$

In brief, 1 mL of *EHEC* (10^6 CFU/mL in 10-fold diluted LB broth) was incubated with 3-armed PLL_{20}-*g*-$Indo_{0.2}$ (7.5 μM) for 1 h, followed by staining with 10 μL 1-N-phenylnaphthylamine (1 mM in acetone) for the labeling of the outer membrane, and 2 μL propidium iodide (1 mg/mL in PBS) to stain the bacterial genome according to previously reported procedures.[1, 2]. Multiphoton excitation microscopy analyses were carried out on an upright optical microscope (Axio imager 2, Carl Zeiss, Germany) with a high-sensitivity EMCCD camera (iXon Ultra 888, Andor, UK) as reported on previous studies.[3]

1. I. M. Helander and T. Mattila-Sandholm, *J Appl Microbiol*, 2000, **88**, 213-219.
2. N. Yamaguchi and M. Nasu, *Journal of Applied Microbiology*, 1997, **83**, 43-52.
3. C. Y. Chang, Y. Y. Hu, C. Y. Lin, C. H. Lin, H. Y. Chang, S. F. Tsai, T. W. Lin and S. J. Chen, *Biomed Opt Express*, 2016, **7**, 1727-1736.

Table S1. Characterization of linear and star poly(Z-L-lysine) (*l*-PZLL and *s*-PZLL) homopolypeptides.

Polypeptide	[initiator] : [ZLL NCA]	^{1}H NMR		GPC-LS		
		DP per arm	Calculated M_n	M_n	M_w/M_n	DP per arm[a]
$PZLL_{20}$	1 : 20	20	5,340	5,900	1.12	22.5
$PZLL_{40}$	1 : 40	38	10,056	10,400	1.20	39.7
3-armed $PZLL_{20}$	1 : 60	20	15,851	17,000	1.37	21.6
4-armed $PZLL_{20}$	1 : 80	19	20,044	24,000	1.43	22.9
6-armed $PZLL_{20}$	1 : 120	23	36,404	35,200	1.42	22.4
8-armed $PZLL_{20}$	1 : 160	32	67,436	63,400	1.56	30.2

[a] DP per arm = number-averaged molecular weight (M_n)/molecular weight of ZLL (262 g/mol)/arm number (n)

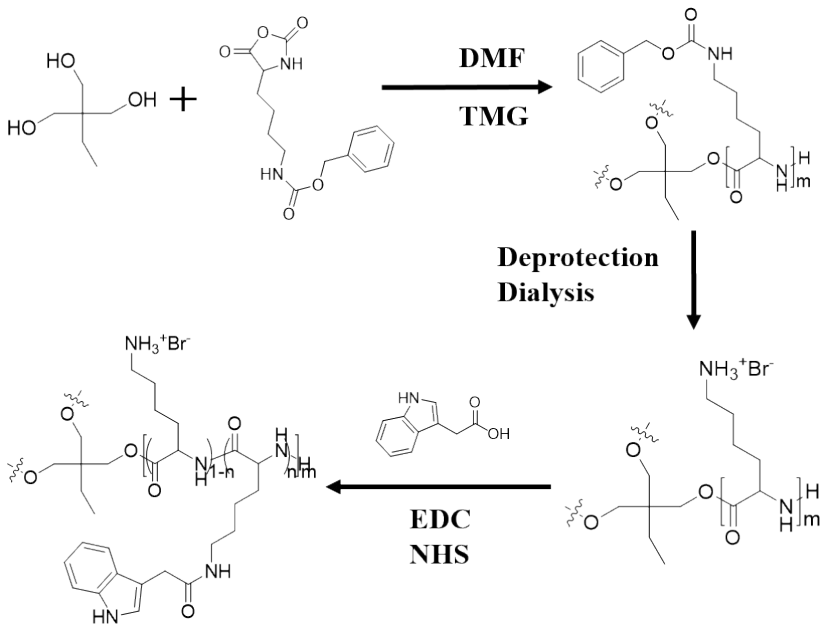
Table S2. Antimicrobial activity of linear and star-shaped homopolypeptides and graft copolypeptides against *E. coli* and *K. pneumoniae* as well as the calculated grafting ratio for graft copolypeptides.

Polypeptide	Grafting ratio (%)	IC_{50} (μM)	
		E. coli	***K. pneumoniae***
PLL_{20}	---	1.96	4.33
PLL_{20}-*g*-$Indo_{0.2}$	20.0	1.36	8.88
PLL_{20}-*g*-$Phenyl_{0.2}$	17.5	1.69	2.03
PLL_{20}-*g*-$Phenol_{0.2}$	17.2	4.57	---
PLL_{20}-g-$Catechol_{0.2}$	21.7	4.36	---
PLL_{40}	---	1.16	2.51
PLL_{40}-*g*-$Indo_{0.2}$	20.4	0.72	2.34
PLL_{40}-*g*-$Phenyl_{0.2}$	22.5	0.97	2.36
3-armed PLL_{20}	---	0.68	1.63
3-armed PLL_{20}-*g*-$Indo_{0.2}$	19.2	0.46	3.03
3-armed PLL_{20}-*g*-$Phenyl_{0.2}$	16.7	0.56	3.09
4-armed PLL_{20}	---	0.54	1.29
4-armed PLL_{20}-*g*-$Indo_{0.2}$	19.2	0.35	2.40
4-armed PLL_{20}-*g*-$Phenyl_{0.2}$	22.7	0.46	2.89
6-armed PLL_{20}	---	0.25	0.35
6-armed PLL_{20}-*g*-$Indo_{0.2}$	23.2	0.19	0.65
8-armed PLL_{20}	---	0.23	---
Ampicillin	---	56.32	54.09

Table S3. LD_{50} values for effects of linear and star-shaped PLL_{20} homopolypeptides and PLL_{20}-*g*-$Indo_{0.2}$ graft copolypeptides on viability of cancer and non-cancer cells.

Polypeptide	LD_{50} (μM)			
	BEAS-2B	**H1299**	**293T**	**TCCSUP**
PLL_{20}	10.31	9.69	9.75	10.16
PLL_{20}-*g*-$Indo_{0.2}$	20.29	16.38	12.88	15.00
3-armed PLL_{20}	4.28	2.11	4.96	5.11
3-armed PLL_{20}-*g*-$Indo_{0.2}$	10.02	5.61	10.19	9.69
6-armed PLL_{20}	2.09	1.24	4.76	4.92
6-armed PLL_{20}-*g*-$Indo_{0.2}$	10.19	5.29	9.67	10.68

LD_{50} value is given as μM of polypeptides concentration determined by WST-8 assay after 24 hours of incubation.



Scheme S1. The representative synthesis of star-shaped PLL-*g*-Indo graft copolypeptide with 3 arms (3-armed PLL-*g*-Indo) using 1,1,1-tris(hydroxymethyl)propane as initiator.

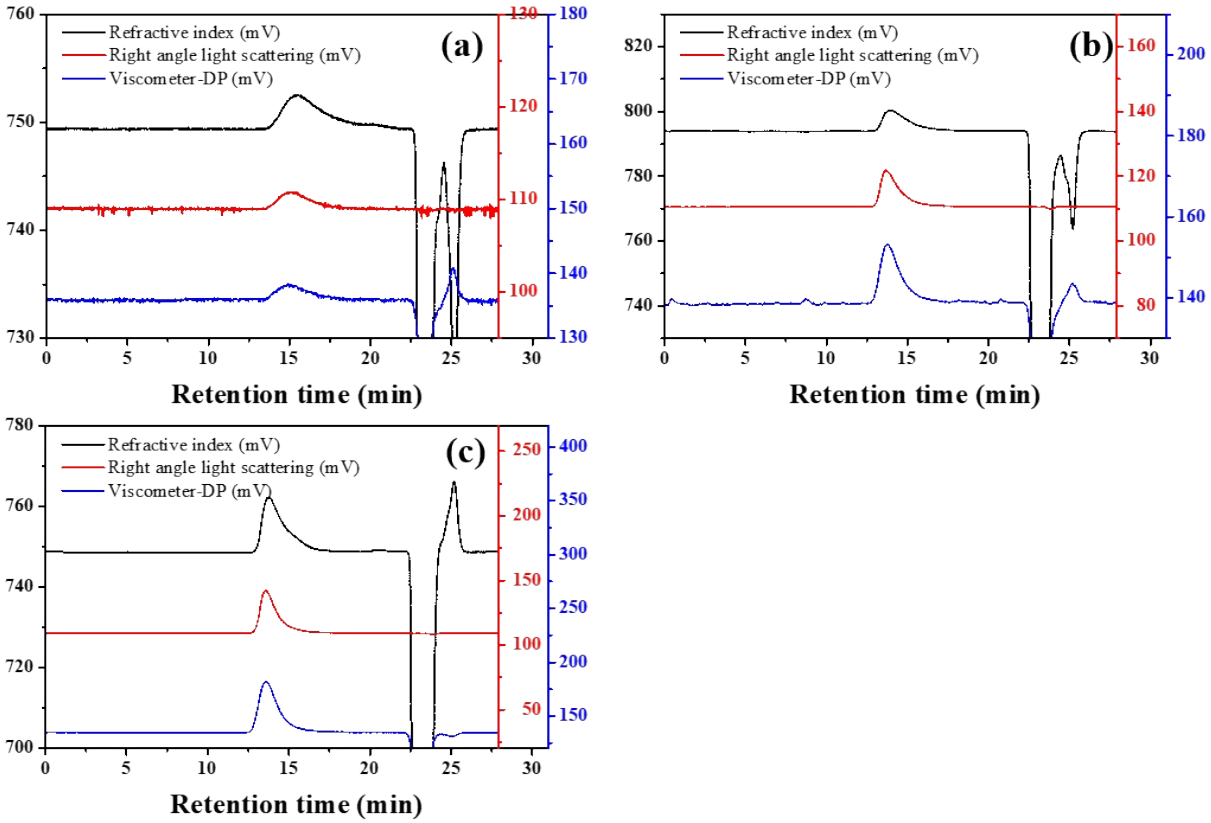


Fig. S1. GPC-LS chromatograms of (a) 3*s*-PZLL$_{20}$, (b) 6*s*-PZLL$_{20}$, and (c) 8*s*-PZLL$_{20}$ star-shaped polypeptides.

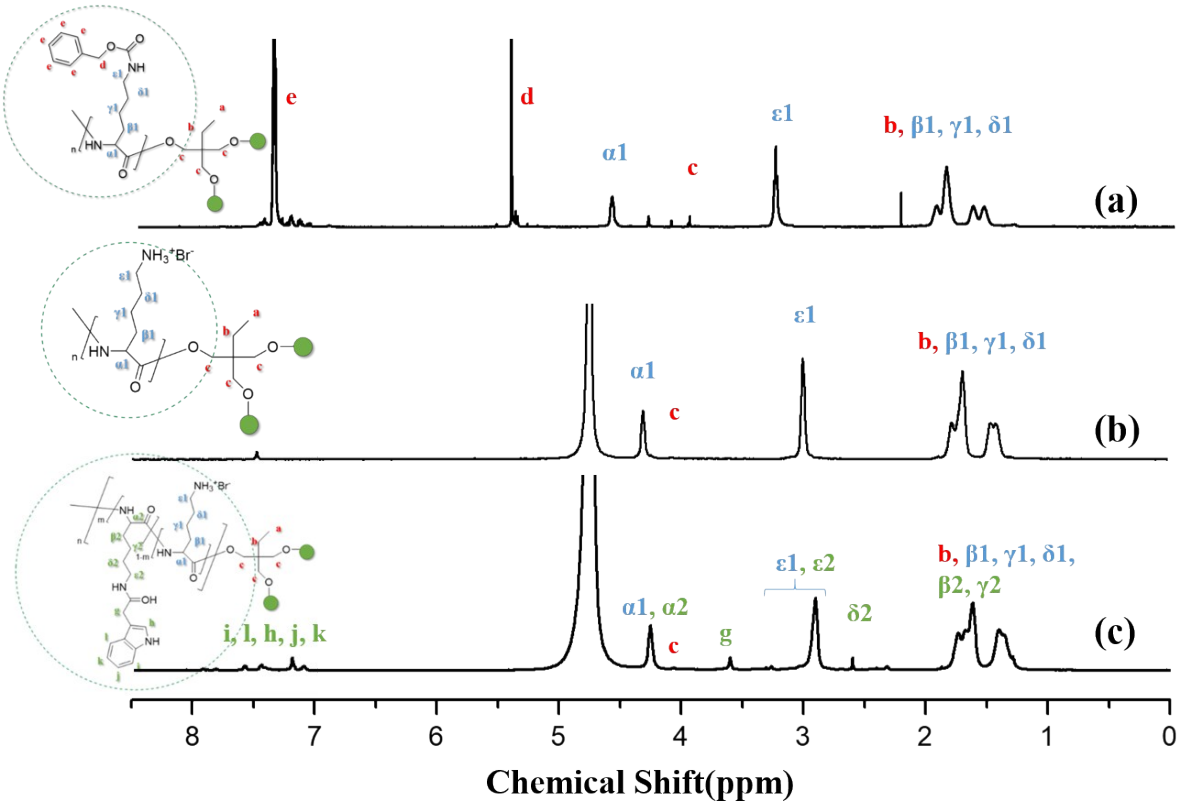


Fig. S2. ^{1}H NMR spectra of (a) 3-armed $PZLL_{20}$ in TFA-d_1, (b) 3-armed PLL_{20} in in D_2O, and (c) 3-armed PLL_{20}-*g*-$Indo_{0.2}$ in D_2O.

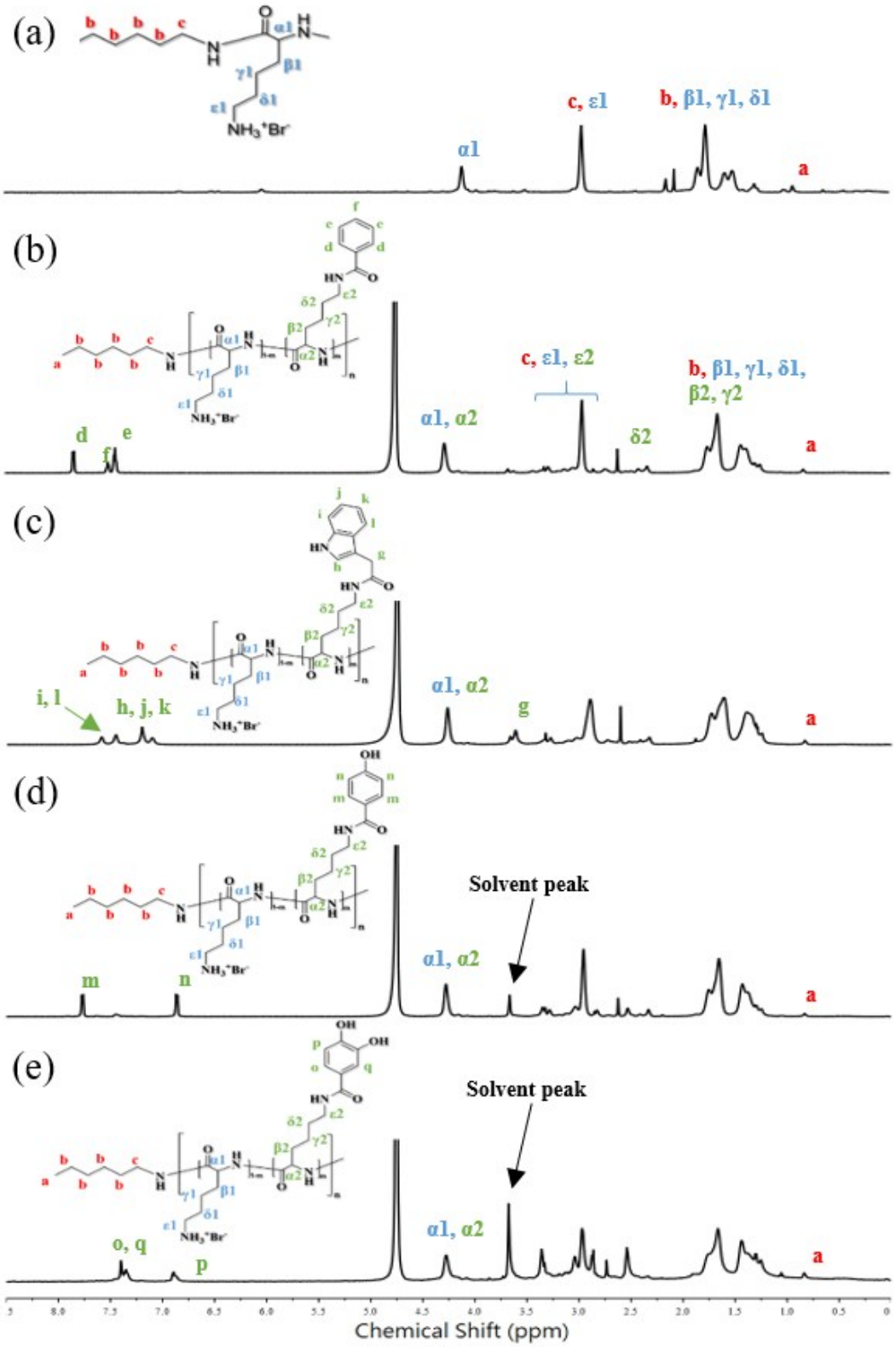


Fig. S3. ^{1}H NMR spectra of (a) $PZLL_{20}$ in TFA-d_1, (b) PLL_{20}-*g*-$Phenyl_{0.2}$ in in D_2O, (c) PLL_{20}-*g*-$Indo_{0.2}$ in D_2O,(d) PLL_{20}-*g*-$Phenol_{0.2}$ in in D_2O, and (e) PLL_{20}-*g*-$Catechol_{0.2}$ in D_2O.

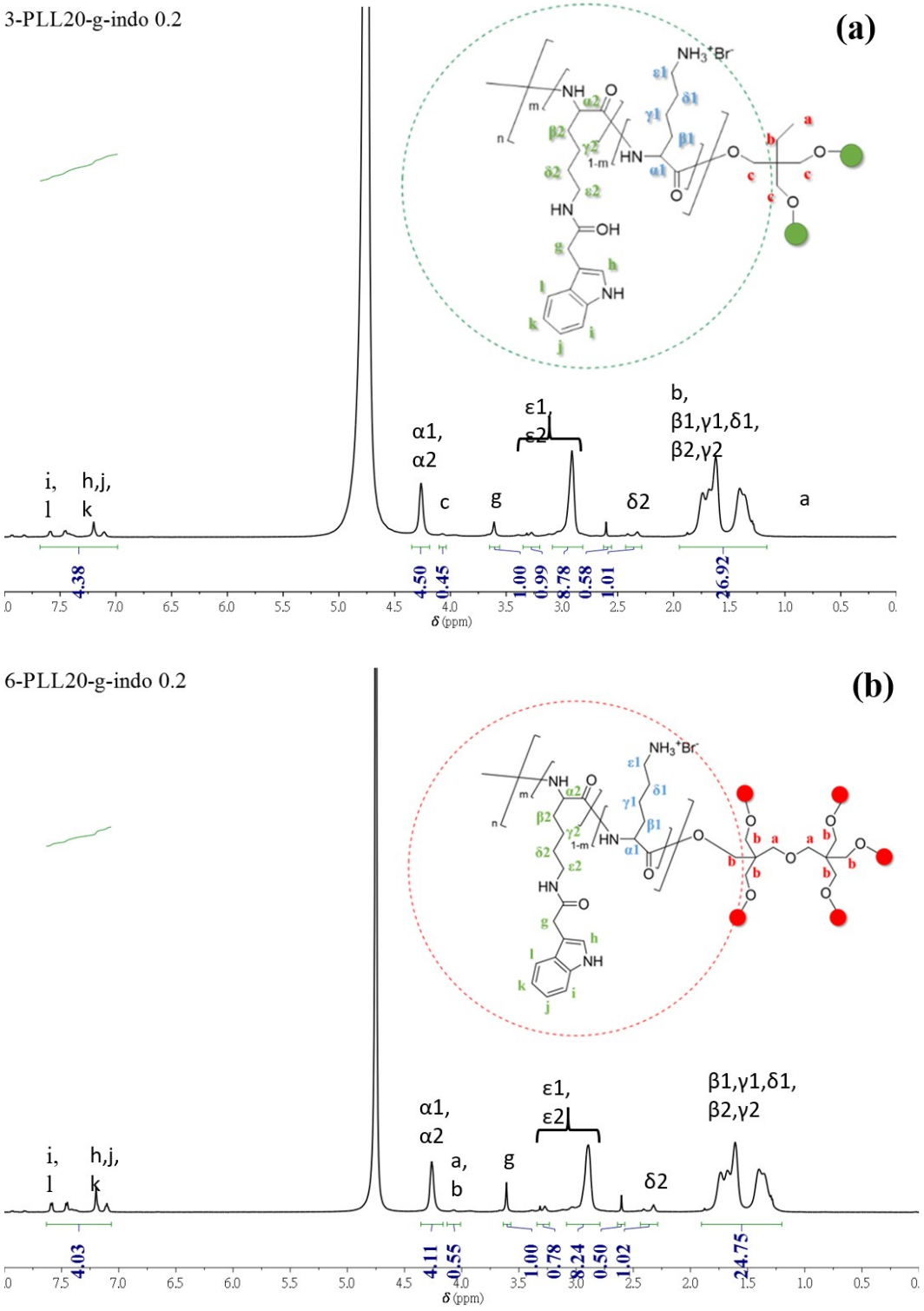


Fig. S4. ^{1}H NMR spectra of (a) 3-armed PLL_{20}-*g*-$Indo_{0.2}$ and (b) 6-armed PLL_{20}-*g*-$Indo_{0.2}$ in D_2O.

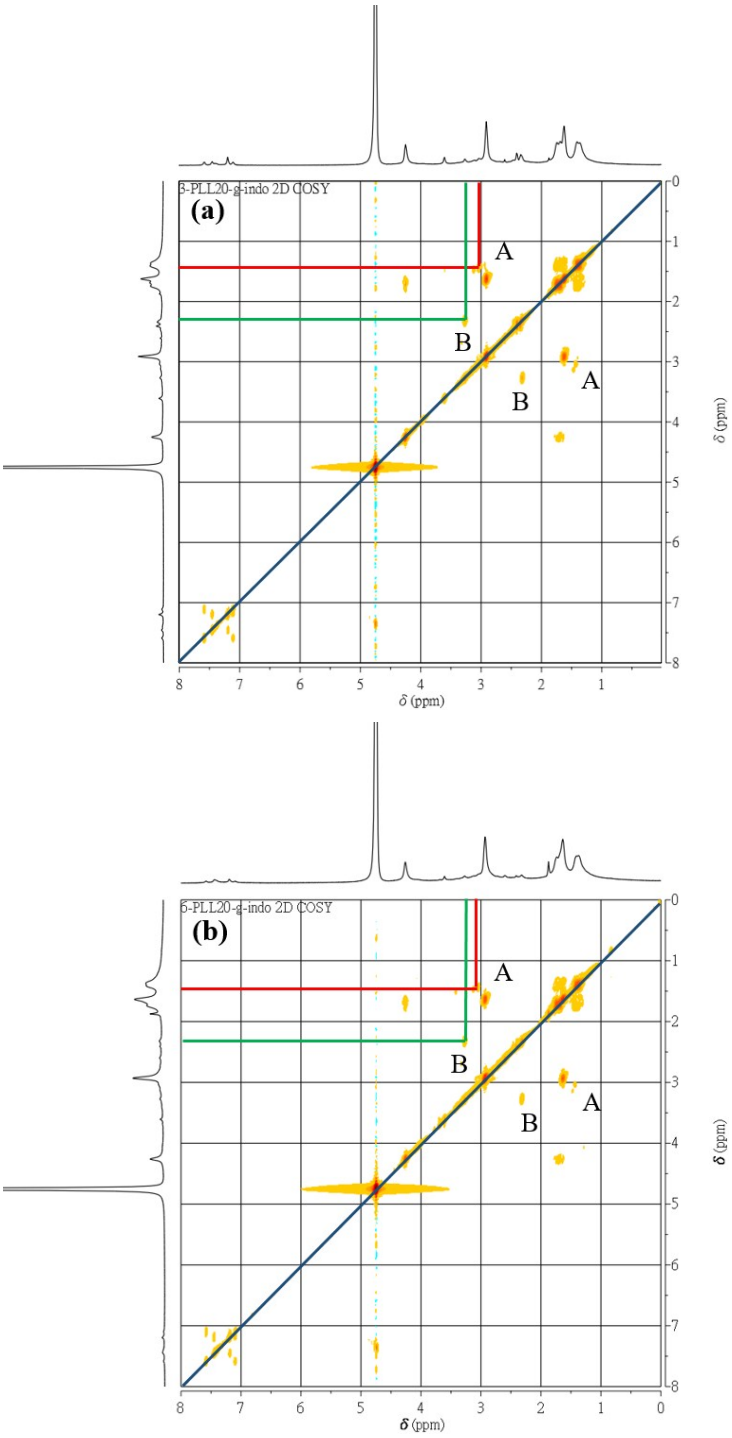


Fig. S5. 2D COSY NMR spectra of (a) 3-armed PLL_{20}-*g*-$Indo_{0.2}$ and (b) 6-armed PLL_{20}-*g*-$Indo_{0.2}$ in D_2O.

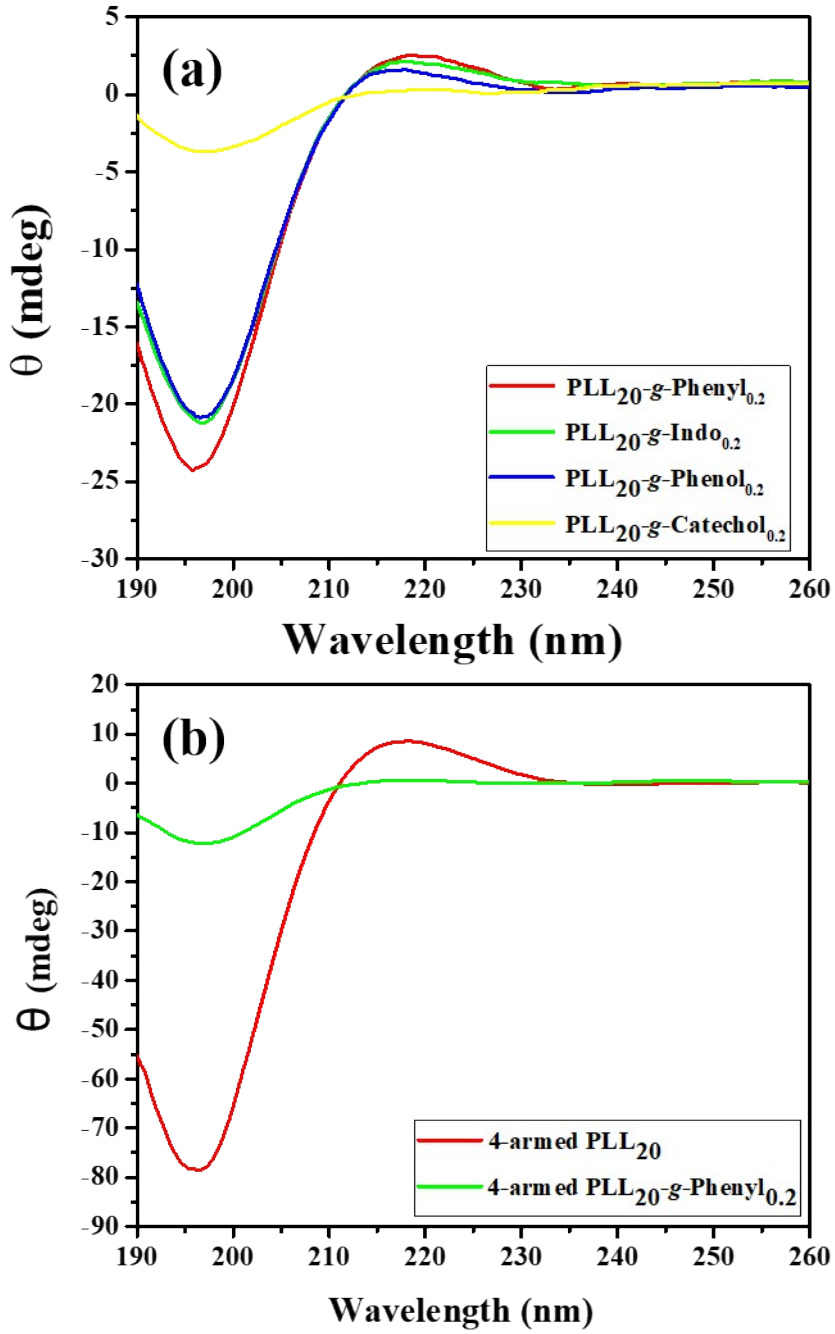


Fig. S6. CD spectra of (a) *l*-PLL$_{20}$-*g*-Phenyl$_{0.2}$, *l*-PLL$_{20}$-*g*-Indo$_{0.2}$, *l*-PLL$_{20}$-*g*-Phenol$_{0.2}$, and *l*-PLL$_{20}$-*g*-Catechol$_{0.2}$ graft copolypeptides (b) 4*s*-PLL$_{20}$ homopolypeptide and 4*s*-PLL$_{20}$-*g*-Indo$_{0.2}$ graft copolypeptide. The polypeptide concentration was 0.25 mg/mL.

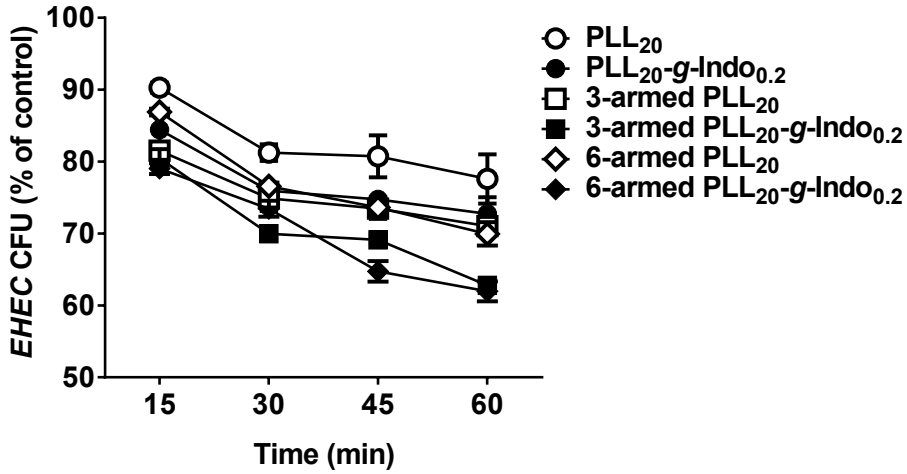


Fig. S7. *EHEC* growth was inhibited by polypeptides in the first hour. *EHEC* (2×10^5 CFU/mL) was incubated with 20 μM polypeptides or vehicle for 15, 30, 45 and 60 min. 100 μL mixture broth was taken out to smear on LB agar plates. Vehicle-treated bacteria were used as control groups.

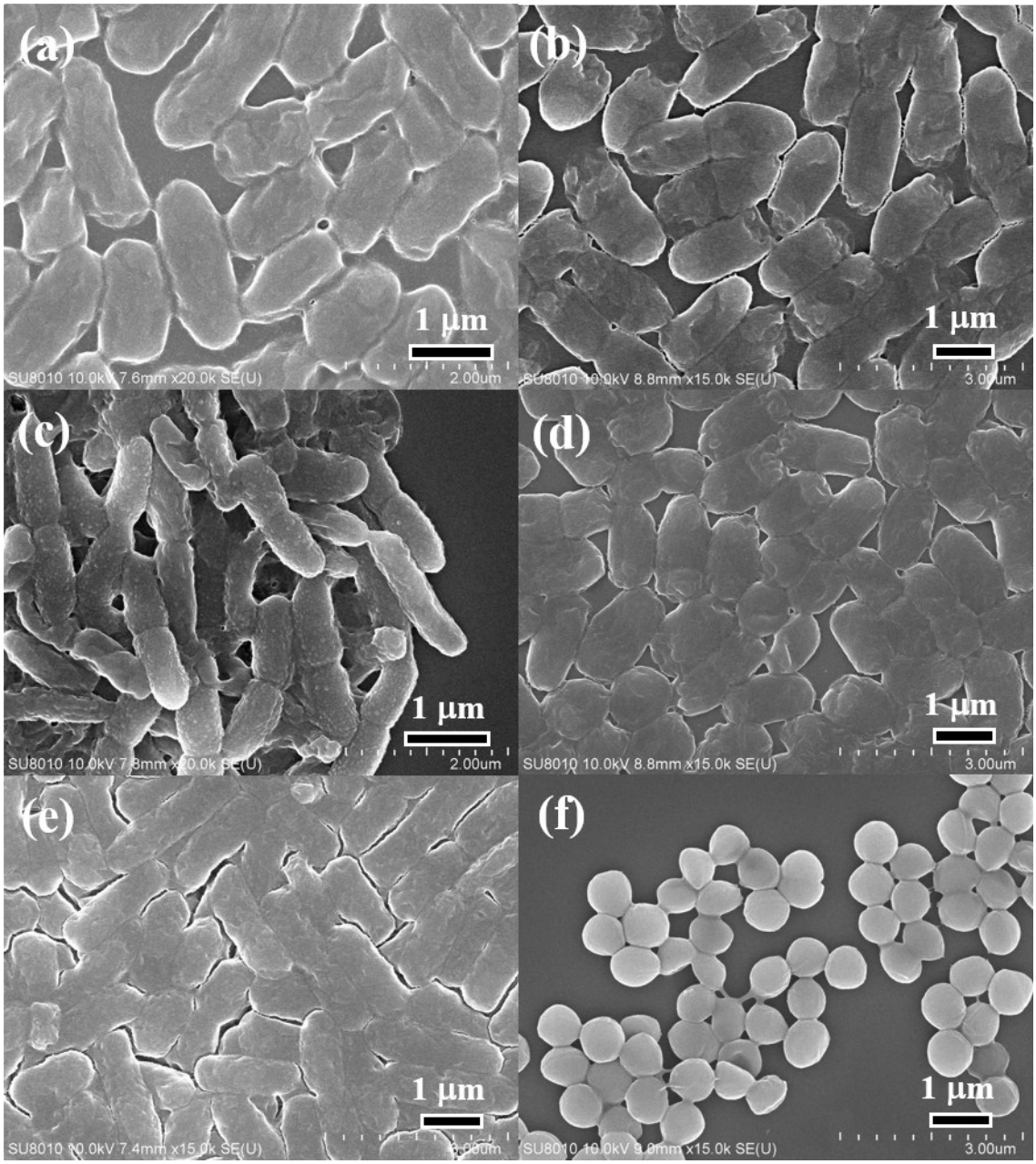


Fig. S8. FE-SEM images of (a) *K. pneumoniae*, (b) *EHEC*, (c) *P. aeruginosa*, (d) *S. sonnei*, (e) *S. typhimurium*, and (f) *S. aureus* bacteria without treatment.

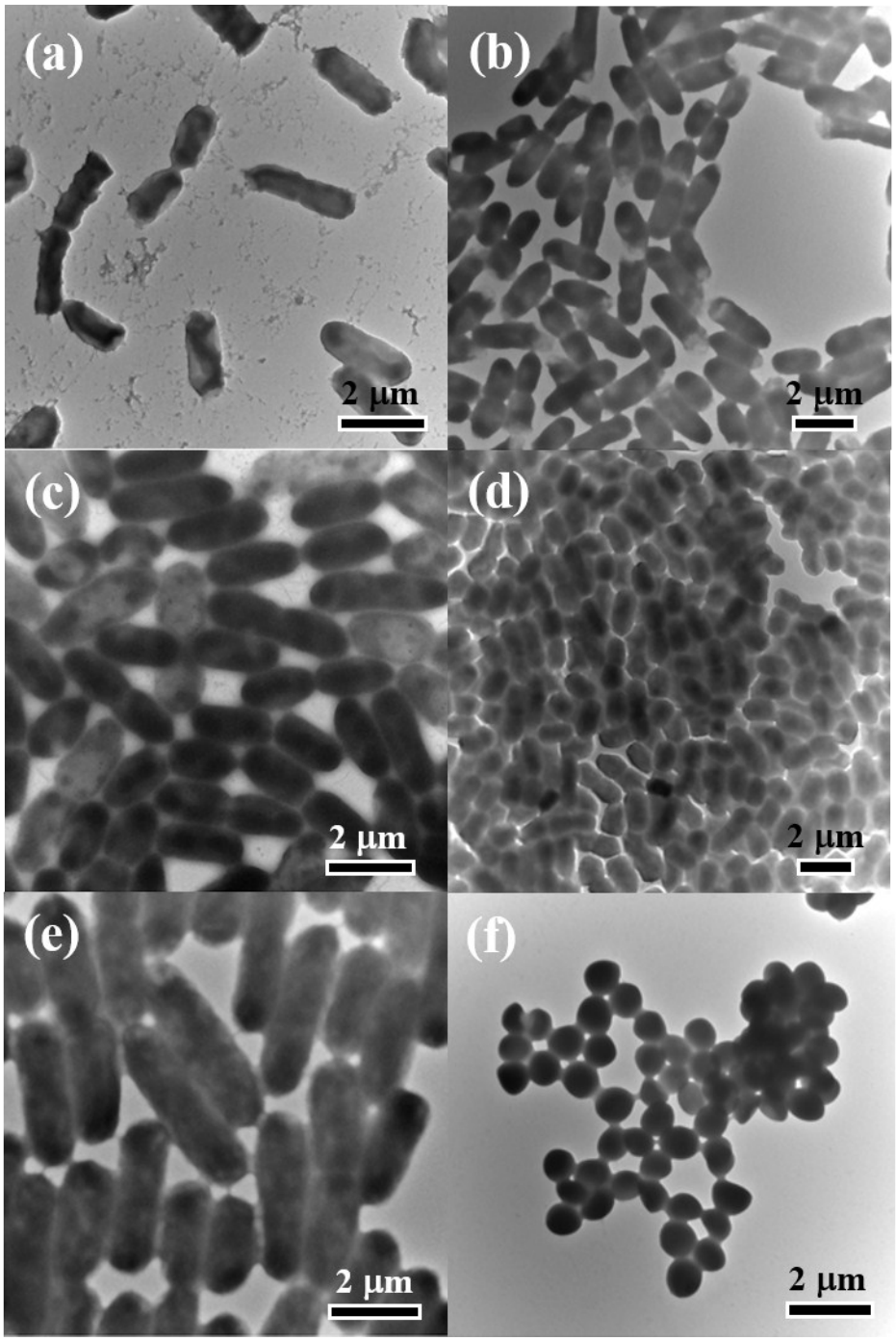


Fig. S9. TEM images of (a) *K. pneumoniae*, (b) *EHEC*, (c) *P. aeruginosa*, (d) *S. sonnei*, (e) *S. typhimurium*, and (f) *S. aureus* bacteria without treatment.

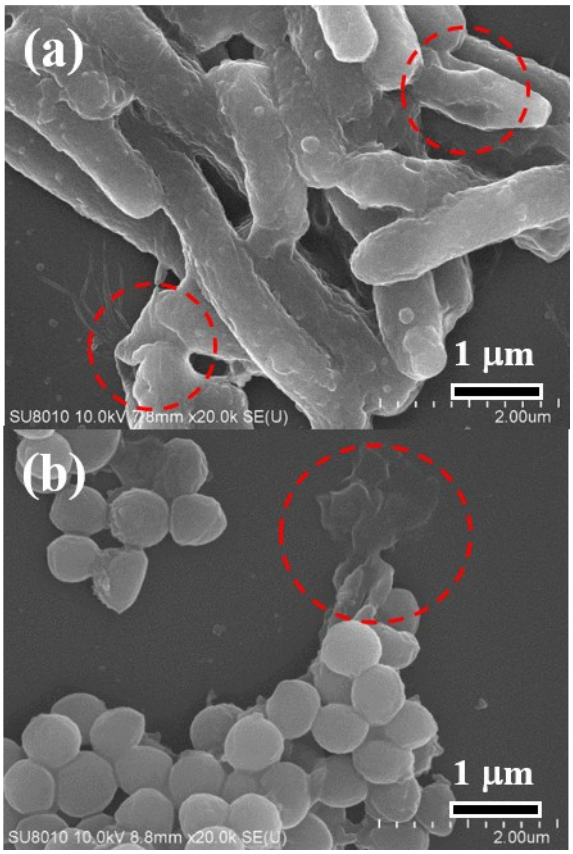


Fig. S10. FE-SEM images of (a) *E. coli* and (b) *S. aureus* bacteria treated with 6*s*-PLL_{20}-*g*-$Indo_{0.2}$ graft copolypeptides for 1 h.

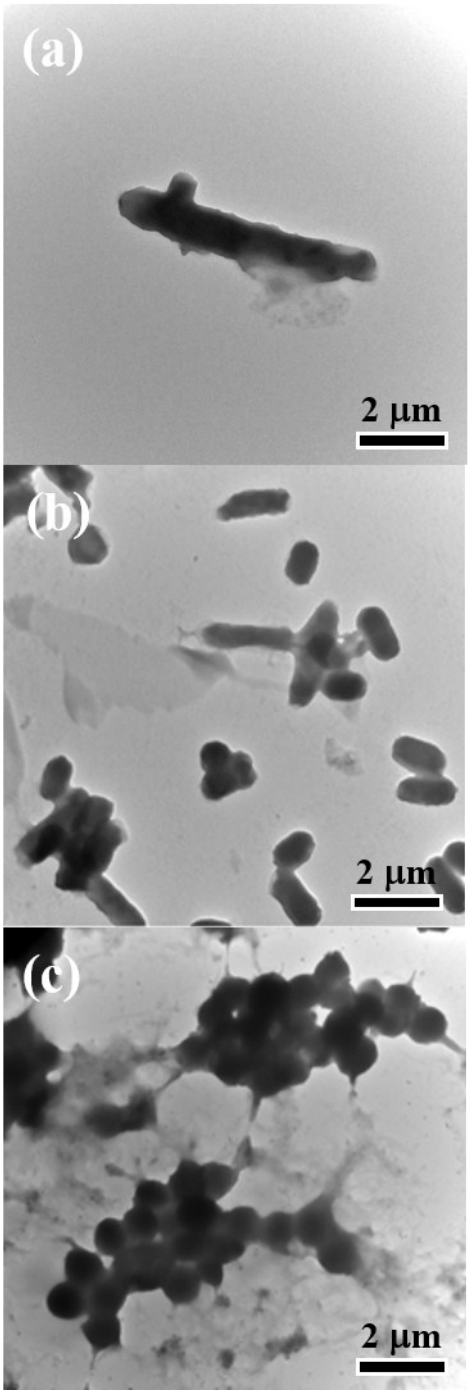


Fig. S11. TEM images of (a) *E. coli*, (b) *S. typhimurium*, and (c) *S. aureus* bacteria treated with 6*s*-PLL_{20}-*g*-$Indo_{0.2}$ graft copolypeptides for 1 h.

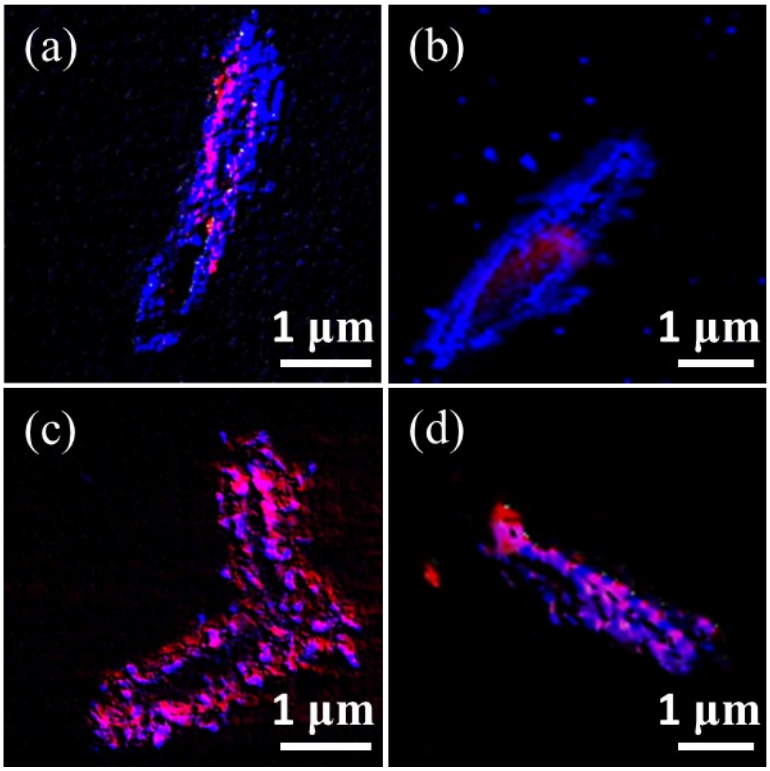


Fig. S12. Three-dimensional (3D) multiphoton images of *EHEC* treated for 1 h with (a) PBS or (c) 3-armed PLL_{20}-*g*-$Indo_{0.2}$. Z-projection multiphoton images of *EHEC* treated for 1 h with (b) PBS and (d) 3-armed PLL_{20}-*g*-$Indo_{0.2}$. 10 μM 1-N-phenylnaphthylamine (NPN, blue fluorescence) and 2 μg/mL propidium iodide (PI, red fluorescence) were used to stain the outer membrane and bacterial genome, respectively.

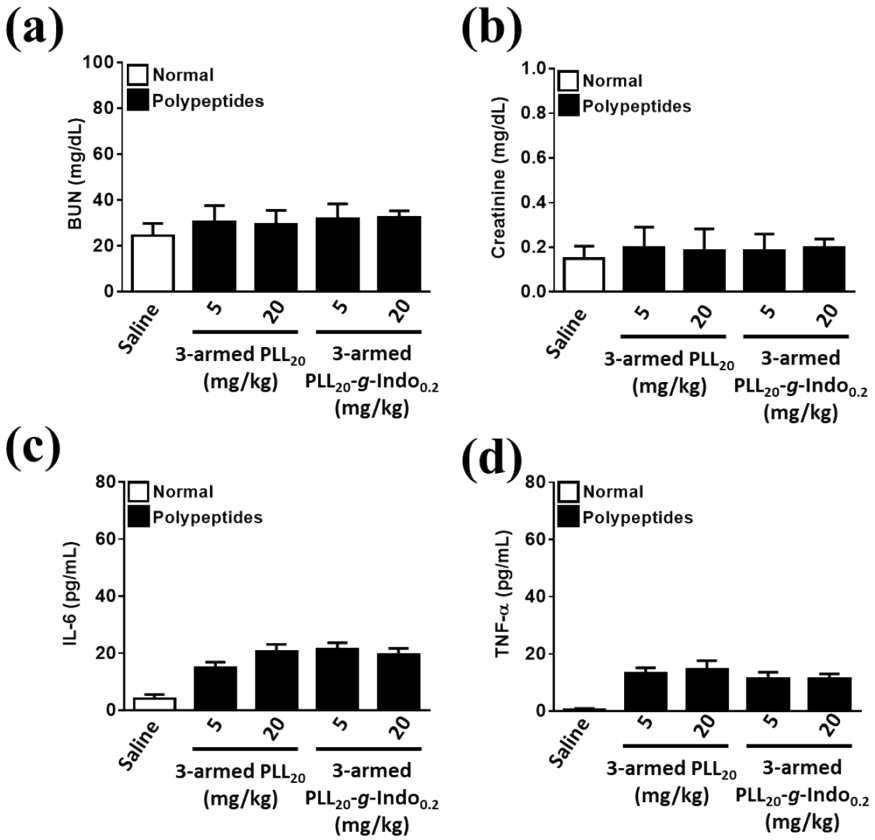


Fig. S13. Treatment of polypeptides alone didn't cause adverse effects on the levels of (a) BUN, (b) serum creatinine, (c) IL-6 and (d) TNF-α. Values are means ± SEM (n = 6).