Supporting Information

Co-ordination Assisted Self-assembled Polypeptide Nanogel to Selectively Combat Bacterial Infection

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1. Synthesis of NCA of Nε-Trifluoroacetyl-L-lysine (Lys(TFA)NCA)

Synthesis of monomer, Lys(TFA) NCA has been done prior to the synthesis of polypeptides with distinct ratios of lysine and valine. In the polypeptide, lysine is supposed to performance as cationic as well as hydrophilic moiety. The triphosgene intermediated NCA of lysine was synthesized according to the procedure mentioned in literature.[1] The ^{1}H NMR spectrum of the synthesized monomer shows (**Figure S1**) signals at 6.40 and 6.12ppm which are signatures of the amine protons near to the trifluoro protecting group (-N**H**-$COCF_3$; a) and another amine proton of five-member NCA ring (-N**H**COO-; b). Other signals at 4.30 and 3.40ppm are attributed to the –C**H**(CO)NH; c and -C$\mathbf{H}_2$-NHCO-; d protons of NCA monomer as designated on the spectrum. Appearance of a number of multiple peaks in the region from 1.20 to 2.0ppm are assigned to the methylene protons (e, f, g, **Figure S1**) of the lysine as designated on the ^{1}H NMR spectrum. Hence, all the appeared resonance signals conclude the successful synthesis of the Lys(TFA)NCA.

2. Synthesis of NCA of DL Valine (Val NCA)

AMPs are the combination of cation and hydrophobic moieties. Hence to mimic the structure of AMP, here we have chosen valine as a hydrophobic segment along with cationic lysine. Synthesis of 'Val NCA' has been done in a similar way as like Lys(TFA)NCA. The ^{1}H NMR spectrum of Val NCA has been displayed in **Figure S2**. Spectrum shows signals at 6.90 and 4.25ppm which are attributed to the –N**H**-; d and methyne (-C**H**-; c) protons of NCA ring. Other signals at 2.26 and 1.15pp are assigned to the methyne (-C**H**-; b) and two methyl groups' protons (-(C$\mathbf{H}_3$)$_2$; a) of Val NCA as designated on the spectrum. All the appeared resonance signals confirmed the successful synthesis of Val NCA.

3. Synthesis of thiol derivative of mannose acetate (M(OAc)$_4$-SH)

The thiol derivative of mannose was synthesized (Scheme S2) by following the reported method.[2] In brief, solid Iodine (0.165g, 0.001 mol.) was added pinch by pinch into the solution of acetic anhydride (15 mL) and mannose (5g, 0.0277 mol.) at 0 ^{o}C, under vigorous stirring. After complete addition of Iodine, the reaction mixture was brought to room temperature and allowed to stir for 3h. The reaction mixture was

quenched with 5mL saturated solution of $Na_2S_2O_3$ and allowed it to stir for 1h. The workup of the reaction mixture was performed with the saturated solution of $NaHCO_3$ and organic part was extracted with EA. The organic layer was dried by evaporating EA. The obtained yellow viscous liquid (without further modification) was used as a starting material for the next step (Scheme S2). For the synthesis of bromo derivative of mannose (Scheme S2), the yellow viscous liquid was dissolved in dry DCM (20mL) and the mixture was cooled down to 0 ^oC. The HBr in 33% acetic acid (16.5mL, 10 equiv.) was slowly added into mannose acetate solution. After the complete addition of HBr solution, the reaction mixture was brought to room temperature and the progress of the reaction was checked by TLC (2:3 = EA/Hexane, R_f = 0.65). After complete consumption of the starting material (approx. 3h), the reaction mixture was diluted with DCM (10mL) and washed with saturated solution of $NaHCO_3$followed by with water. Organic part was dried over Mg_2SO_4 and concentrated by evaporating the solvent. The bromine derivative of mannose was used for the next step without further purification. Finally, to synthesize the thiol derivative of mannose (Scheme S2), the bromo derivative of mannose was dissolved in dry acetone (50mL) and the solution was added in to the RBF containing activated molecular sieves (4 A^o) and thiourea (3.16g, 0.041mol.). The reaction mixture was continued at reflux condition for 3h under N_2 atmosphere. The reaction mixture was cooled down to room temperature and filtered through Celite. The filtrate was evaporated, and the crude material was re-dissolved in DCM (50mL). An aqueous solution of $Na_2S_2O_5$ (5g in 50mL) was added into the DCM solution of crude material and refluxed for another 1h under N_2 atmosphere. The reaction mixture was cooled down to room temperature and the organic layer was separated out followed by washing with H_2O and saturated NaCl solution. The Mg_2SO_4 dried organic layer was concentrated under vacuum and product was purified by silica gel column using 2:3 = EA/hexane as an eluent (R_f= 0.5). Yield: 82%

4. FESEM sample preparation

The E. coli (EC 8729) bacteria were subculture overnight under LB media.[3] The bacteria suspension was diluted with 1X PBS to achieve 5×10^6 $CFUmL^{-1}$. Bacterial suspension (1mL) was then introduced into 1mL PBS solution of nanoparticle (10mg/mL) and

incubated for 1h at 37 ºC on a horizontal shaker (at 230rpm). The nanoparticle adsorbed bacteria were spin down (rpm 5k for 5min.) and wash with PBS (thrice) followed by took off the supernatant. The sample was fixed with 2.5% glutaraldehyde at 4 ºC, overnight. The sample was then dried by sequential treatment of 50%, 70%, 80% and 100% ethanol. A drop of sample with 100% ethanol was placed on silicon wafer and allowed to dry under room temperature. The interaction of PNG with *E. coli* was captured under FESEM (Merlin, Germany).

5. Cryo-TEM sample preparation

The nanostructure at a concentration 2×MIC and E. coli with 5×10^6 $CFUmL^{-1}$ were incubated together in MHB media according to the procedure as mentioned inthe MIC protocol (section 4.10). After 3h of incubation at 37 ºC, the bacterial cells were pelleted followed by washing with PBS, thrice. Finally, the bacterial pellet was resuspended into PBS. PBS dispersed bacterial suspension was used to prepare cryo-TEM sample by adopting identical procedure as described by Chen et al.[3] The images were captured under Titan Krios transmission electron microscope (working at 300 kV).

6. Experimental Techniques

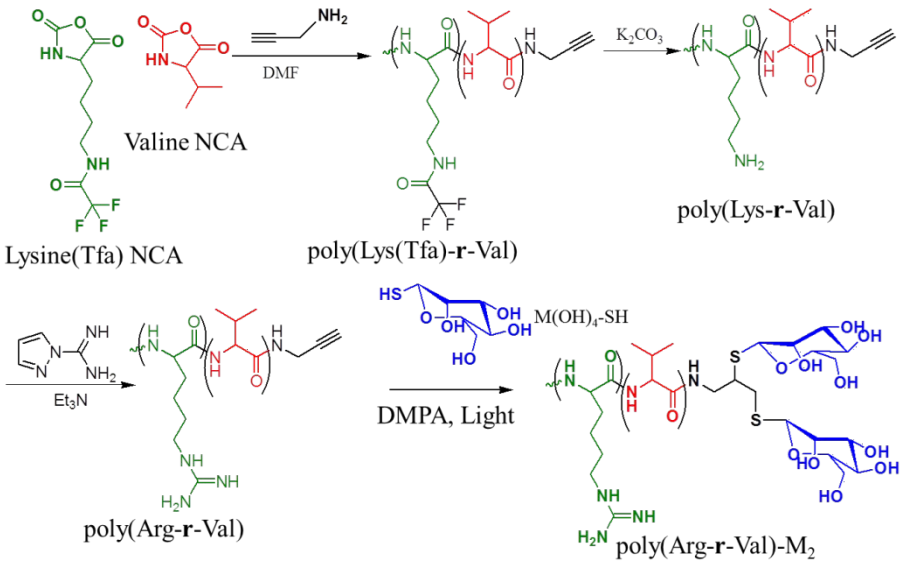
The chemical structure of the synthesized copolymers and their thiol derivative of mannose were characterized by using ^1H and ^{13}C NMR (400 MHz, Bruker Advance Instrument). PerkinElmer FTIR spectrophotometer was used to record the spectrum of the reaction mixture (during polymerization) by using KBr pellet as a support. The molecular weight of the synthesized polymer was determined by Waters GPC containing 2410 refractive index detector (RID). Acetate buffer at the pH 4.5 (@40 °C) was used as eluent and the flow rate was maintained to 1mL/min. The GPC samples were prepared by dissolving polymer into acetate buffer at a concentration of 2mg/mL. For polymer, shifting of absorption band during metal-polymer complex formation was collected by using PerkinElmer's Lambda 35 UV-visible spectra-meter (slit with 1nm). The hydrodynamic size of the fabricated nanostructure was determined by Malvern Nano ZS instrument with a fixed detector position (at 90° angle). He-Ne laser (power = 4 mW) was used as light source to record the date at 25 °C. The surface morphology of the fabricated nanostructure was captured under FESEM (Merlin, Germany) working at

5kV (voltage). HRTEM (JEOL 2000) at 200kV was used to inspect the bulk morphology of fabricated PNG. A thick layer of sample was developed on glass side and subjected to XPS. The source of 'Al' Kα X-ray with the voltage of 1486.7eV photons was used to analyze the sample. The data analysis was performed by choosing C1s at 284.6eV as the reference peak.

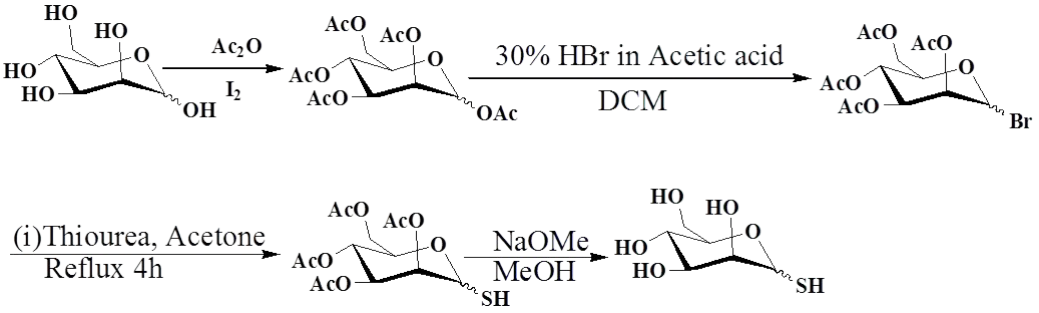
7. Serum Stability

The stability of the PNG in presence of FBS (10%) has been done over 24h. The stability of the PNG was evaluated in terms of their size changes (determined by DLS) after the incubation with FBS serum. The PNG with a concentration of 1mg/ml was incubated with 10% FBS. The effective change of size has been monitored by DLS over the period of 0, 6, 12 and 24h, respectively.

Scheme S1: Scheme for the synthesis of polypeptide, poly(Arg-r-Val)-M_2



Scheme S2: Scheme for the synthesis of thiol derivative of mannose, $M(OH)_4$-SH



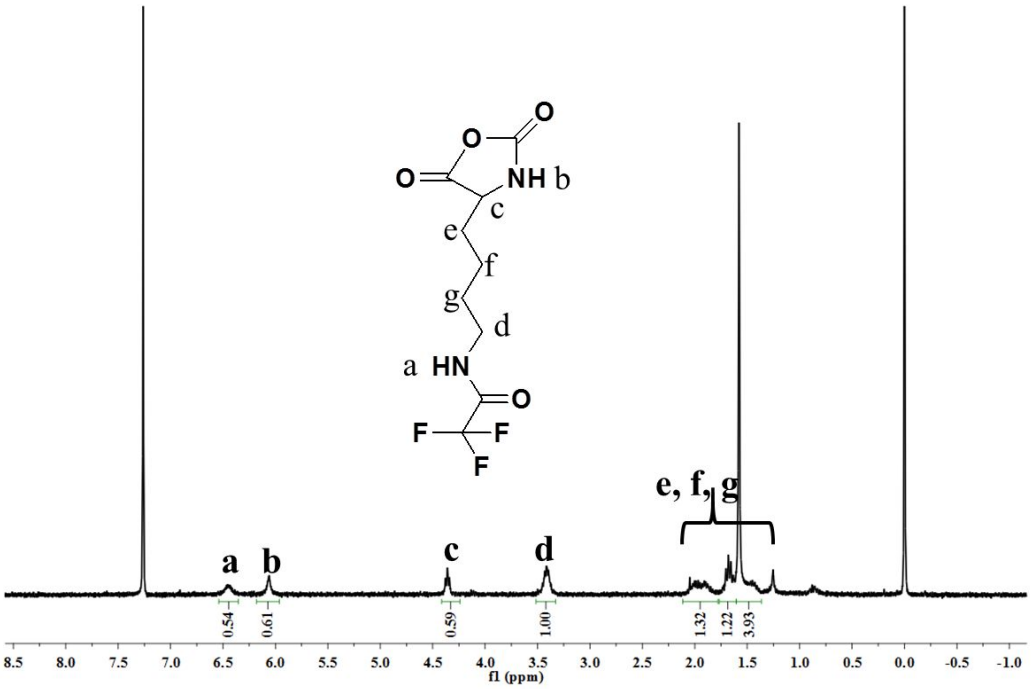


Figure S1: ^{1}H NMR of NCA of lysine(Tfa) in $CdCl_3$

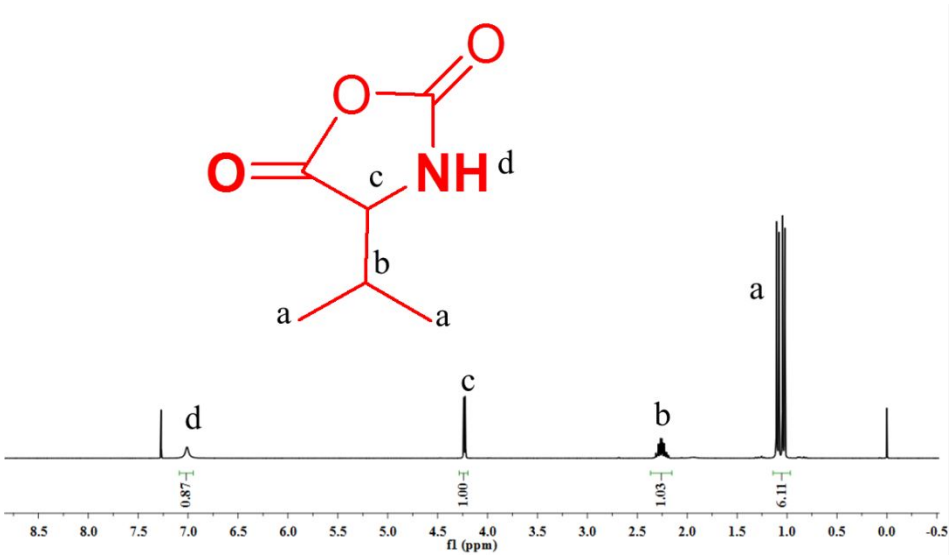


Figure S2: ^{1}H NMR of NCA of valine in $CdCl_3$

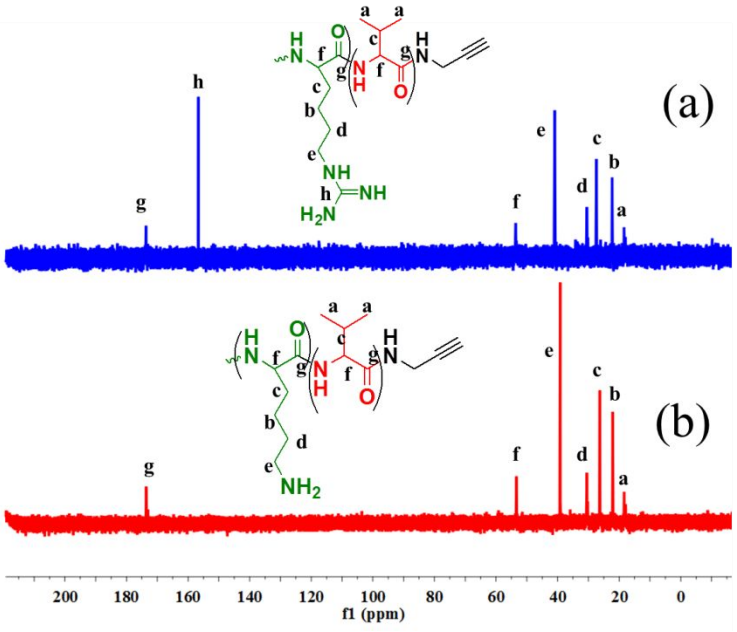


Figure S3: ^{13}C NMR of (a) poly(Arg-r-Val)-M_2 and (b) poly(Lys-r-Val)-M_2

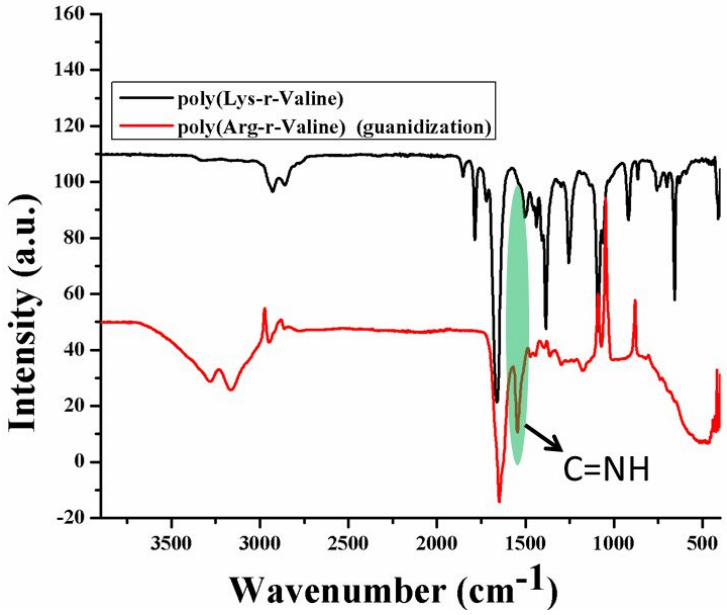


Figure S4: FTIR spectra of poly(lys-r-Val) and poly(Arg-r-Val)

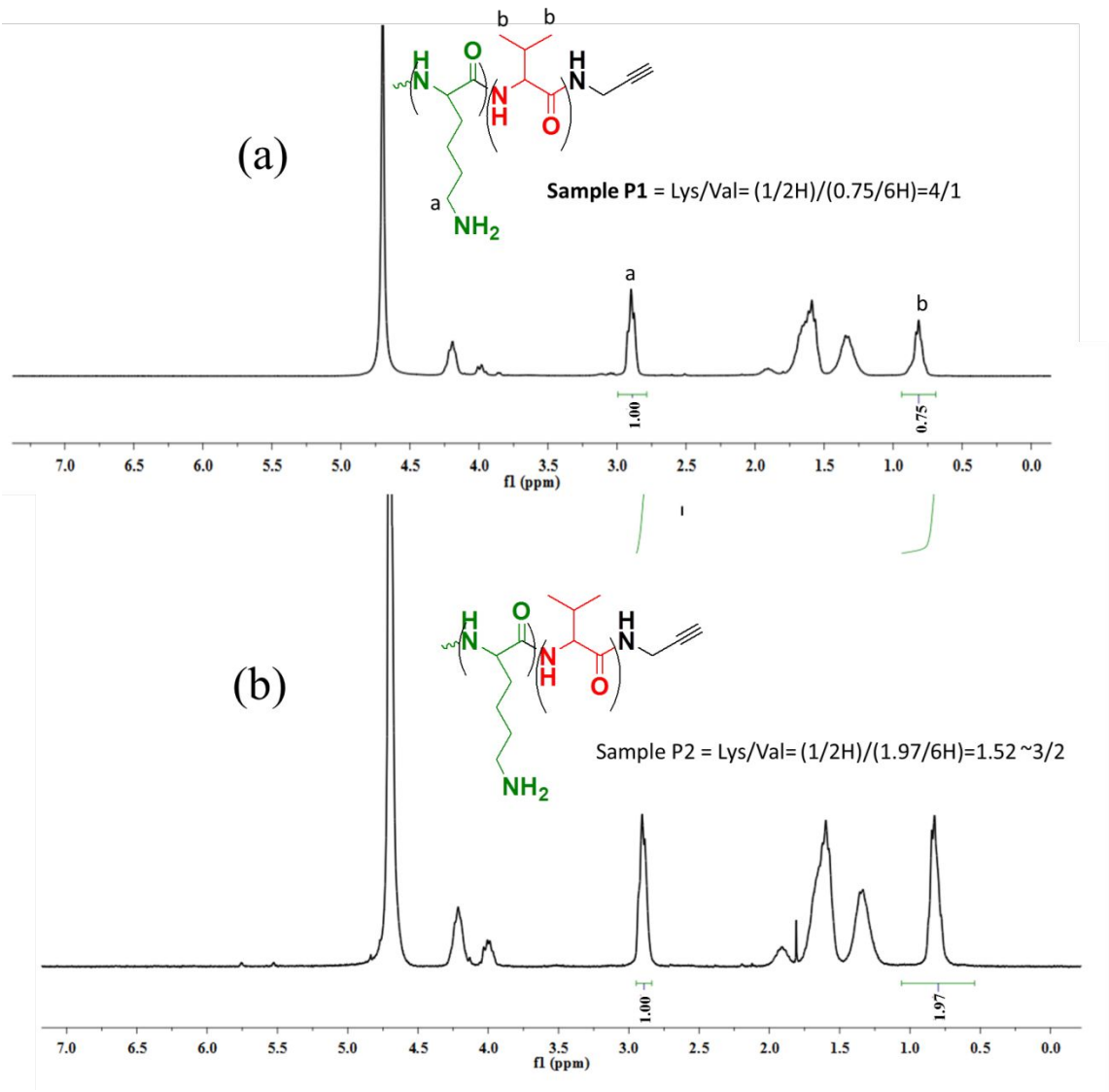


Figure S5: ^{1}H NMR of (a) polypeptide P1 and (b) P2 with their calculated composition of lysine and valine

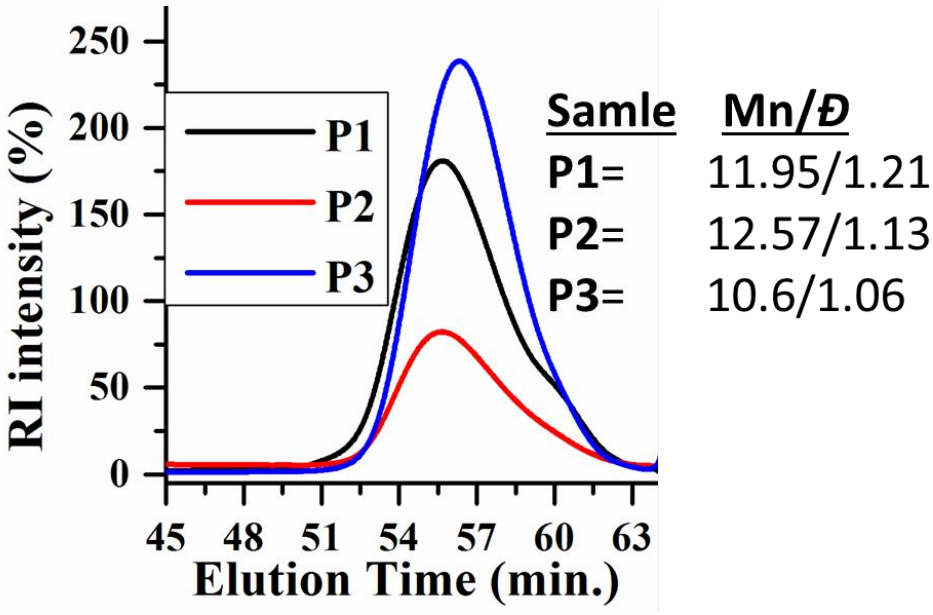


Figure S6: GPC traces of the synthesized polypeptides, P1, P2 and P3 by using water as an eluent.

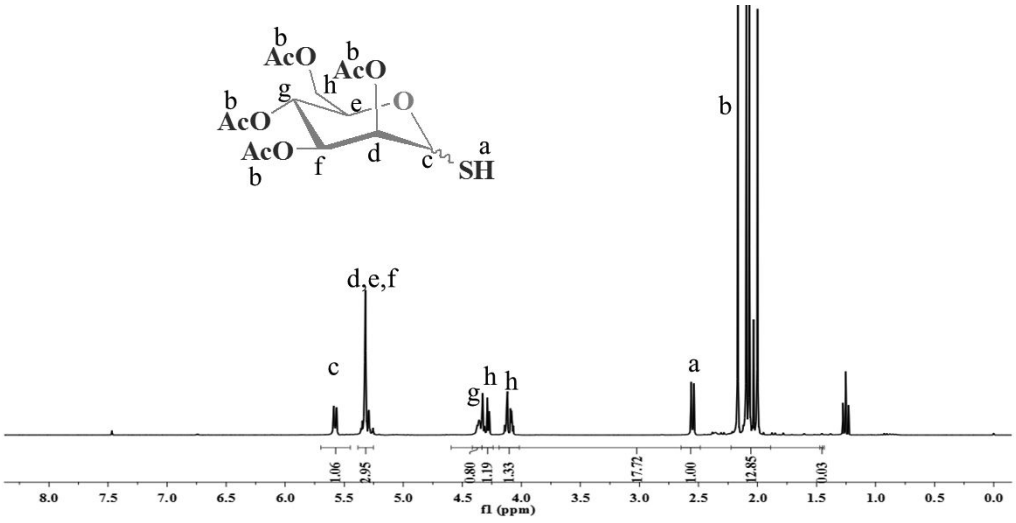


Figure S7: ^{1}H NMR spectrum of the acetyl derivative of thio-mannose, $M(OAc)_4$-SH in $CdCl_3$

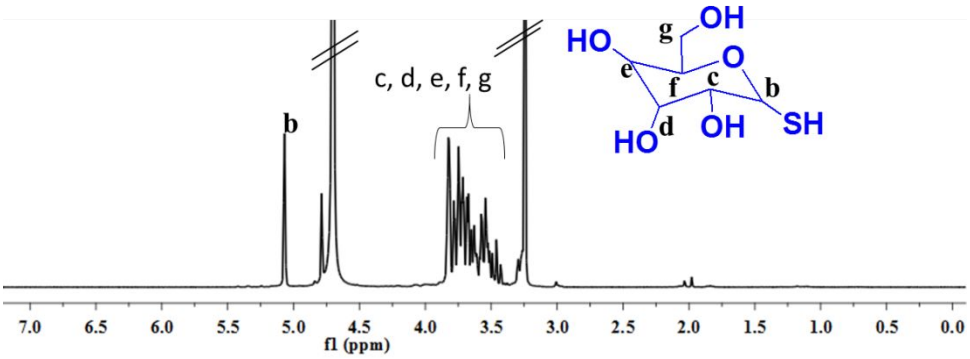


Figure S8: ^{1}H NMR spectrum of thiol derivative of mannose, $M(OH)_4$-SH in D_2O

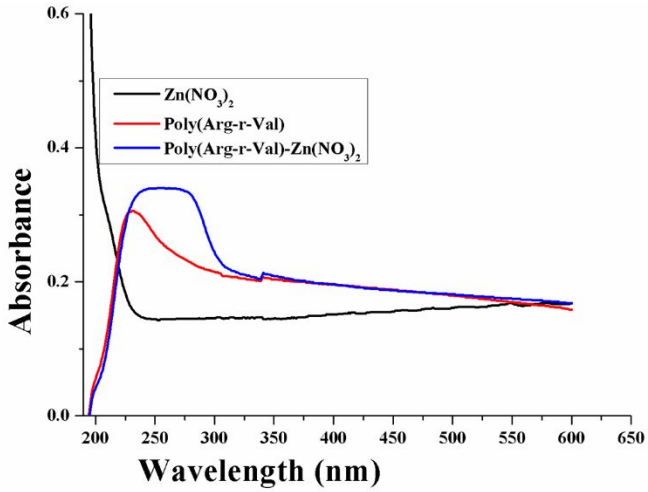


Figure S9: UV-Vis spectra of polypeptides before and after the PNG formation with $Zn(NO_3)_2$

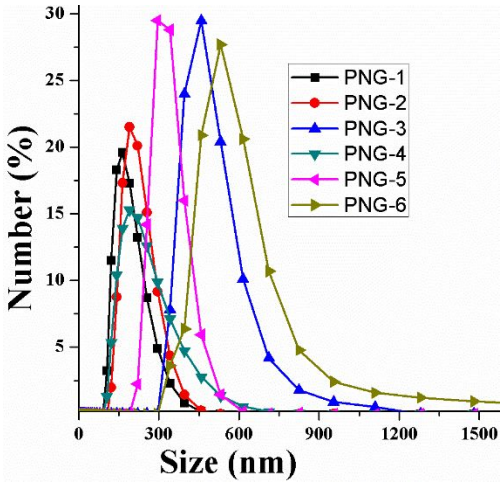


Figure S10: DLS spectrum of the fabricated nanogel

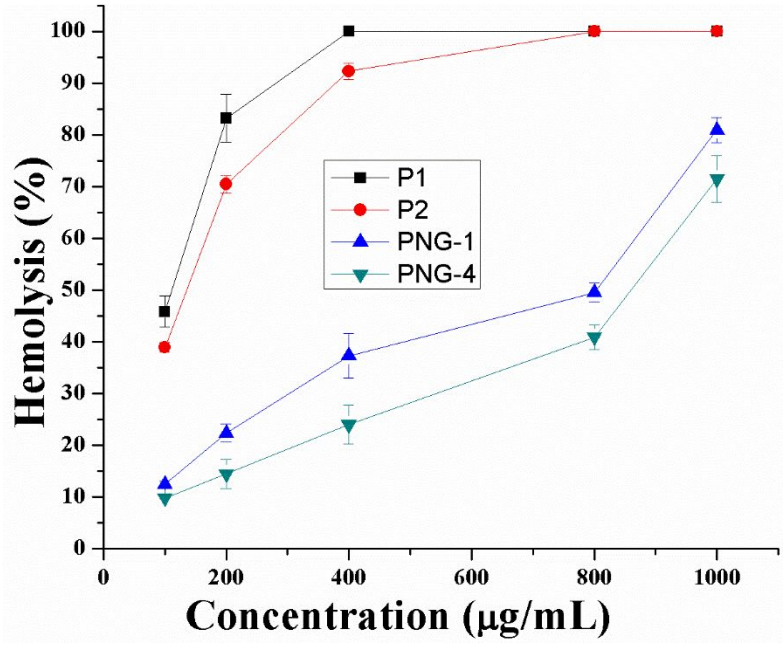


Figure S11: Hemolysis activity of the sample P1, P2, PNG-1 and PNG-4 with increasing the concentration.

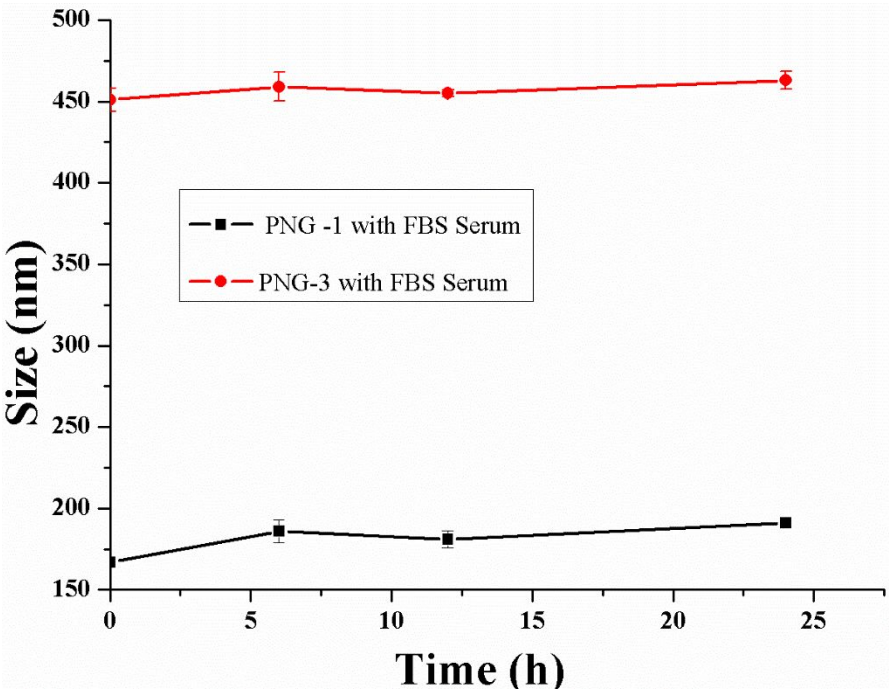


Figure S12: Stability of PNG in the presence of FBS serum over the period of 24h.

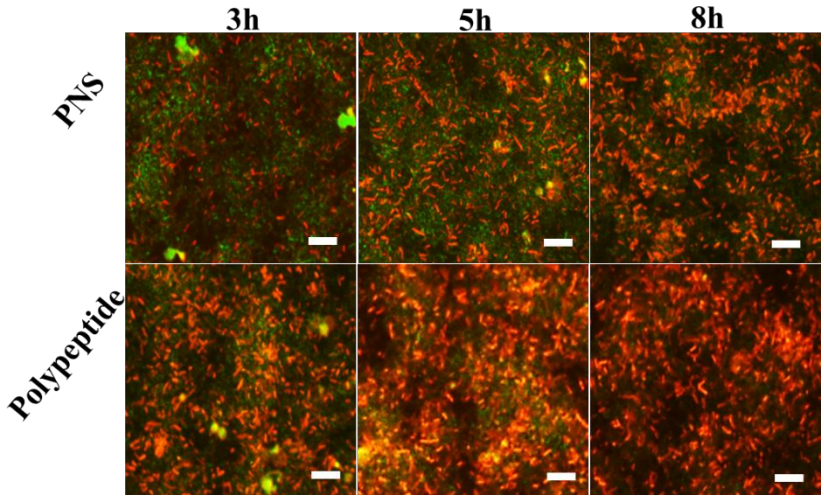


Figure S13: Live-dead assay of the E. coli bacteria treated with polypeptide and PNG at the time frame of 3h, 5h and 8h. The scale bar is 20μm

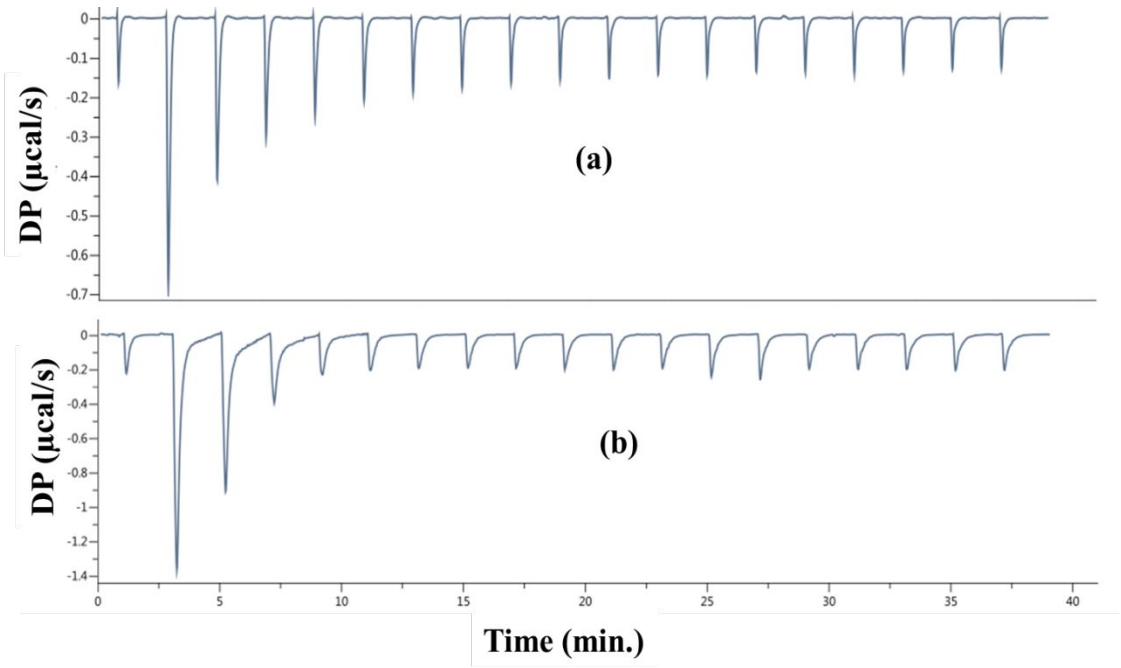


Figure S14: ITC titration curve for poly(Arg-r-Val)-M_2 treated (a) mammalian cell mimicking liposome and (b) bacterial cell mimicking liposome.

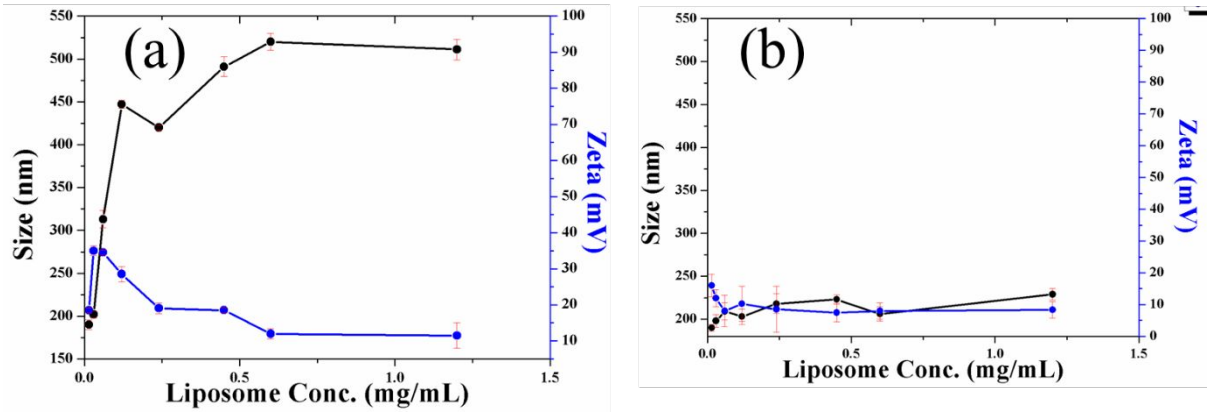


Figure S15: Variation of particle size and zeta potential of PNG with the (a) gram negative bacteria mimicking liposome and (b) Mammalian cell mimicking liposome concentration.

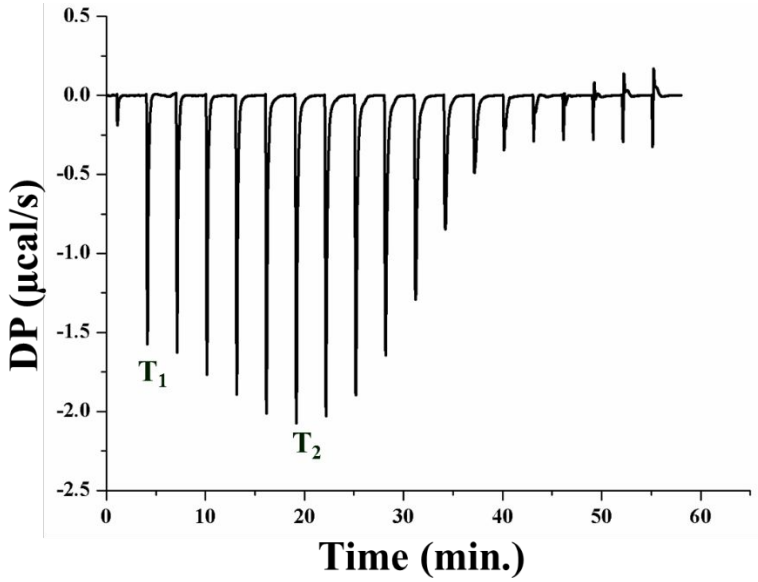


Figure S16: ITC thermogram of the titration of bacterial suspension (1×10^3 CFU/mL) against PNG.

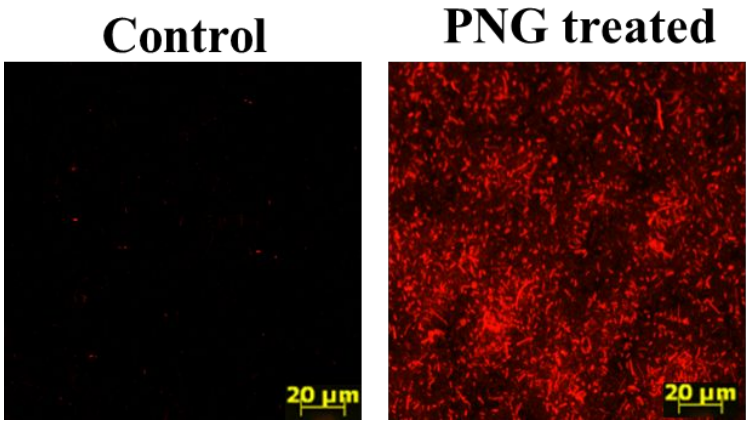


Figure S17: Microscopic image of control and PNG treated *E. coli* after propidium iodide (PI) assay.

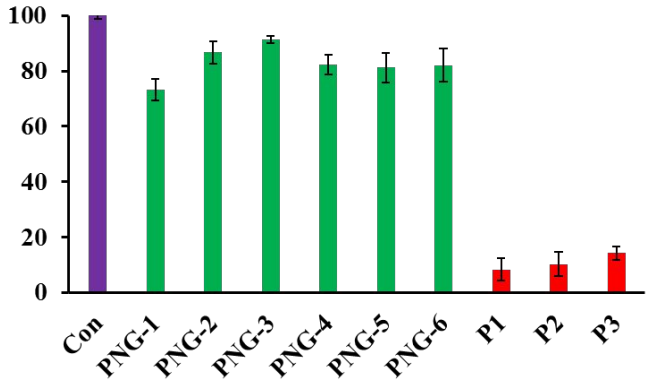


Figure S18: The cell viability assay of the polypeptide and PNG at the concentration of 200 µg/mL.

Table S1: The summery of biological performance of the polypeptide and PNG

Sample	3T3 cell viability (%)		Hemolysis (%)
	@100 µg/mL	@200 µg/mL	@100 µg/mL
PNG-1	79.23 ± 5.1	73.26 ± 3.9	12.38 ±0.56
PNG-2	89.85 ± 3.2	86.71 ± 4.1	11.26 ±5.6
PNG-3	93.26 ± 1.9	91.21 ± 1.3	8.29 ±3.9
PNG-4	86.98 ± 5.6	82.32 ± 3.6	9.37 ±2.3
PNG-5	83.23 ± 3.2	81.25 ± 5.3	10.11 ±2.7
PNG-6	96.36 ± 1.9	82.1 ± 6.1	10.36 ±1.9
P1	14.81 ± 3.1	8.32 ± 4.1	48.86 ± 2.1
P2	24.81 ± 3.6	10.19 ± 4.4	37.27 ± 3.2
P3	19.36 ± 1.8	14.23 ± 2.4	31.28 ±4.3

Table S2: The summery of MIC results obtained against the resistant strain of gram-positive *MRSA* and gram-negative *E. Coli*.

Samples	MIC (μg/ml)	
	MRSA (resistant strain)	***E. coli*** (resistant strain)
P1	2-4	4
P2	4	4-8
P3	16	16-32
PNG-1(P1)	4	4-8
PNG-3(P1)	8	16
PNG-4(P2)	4-8	8
PNG-6(P2)	16	32

Reference

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