Supporting Information for

Chain-Folding Regulated Self-Assembly, Outstanding Bactericidal Activity and Biofilm Eradication by Biomimetic Amphiphilic Polymers

Ranajit Barman,[a] Debes Ray, [b] V. K. Aswal [b] and Suhrit Ghosh* [a]

[a] School of Applied and Interdisciplinary Sciences, Indian Association for the Cultivation of Science, 2A and 2B Raja S. C. Mullick Road, Kolkata, India-700032

[b] Solid State Physics Division, Bhabha Atomic Research Centre, Mumbai 400085, India

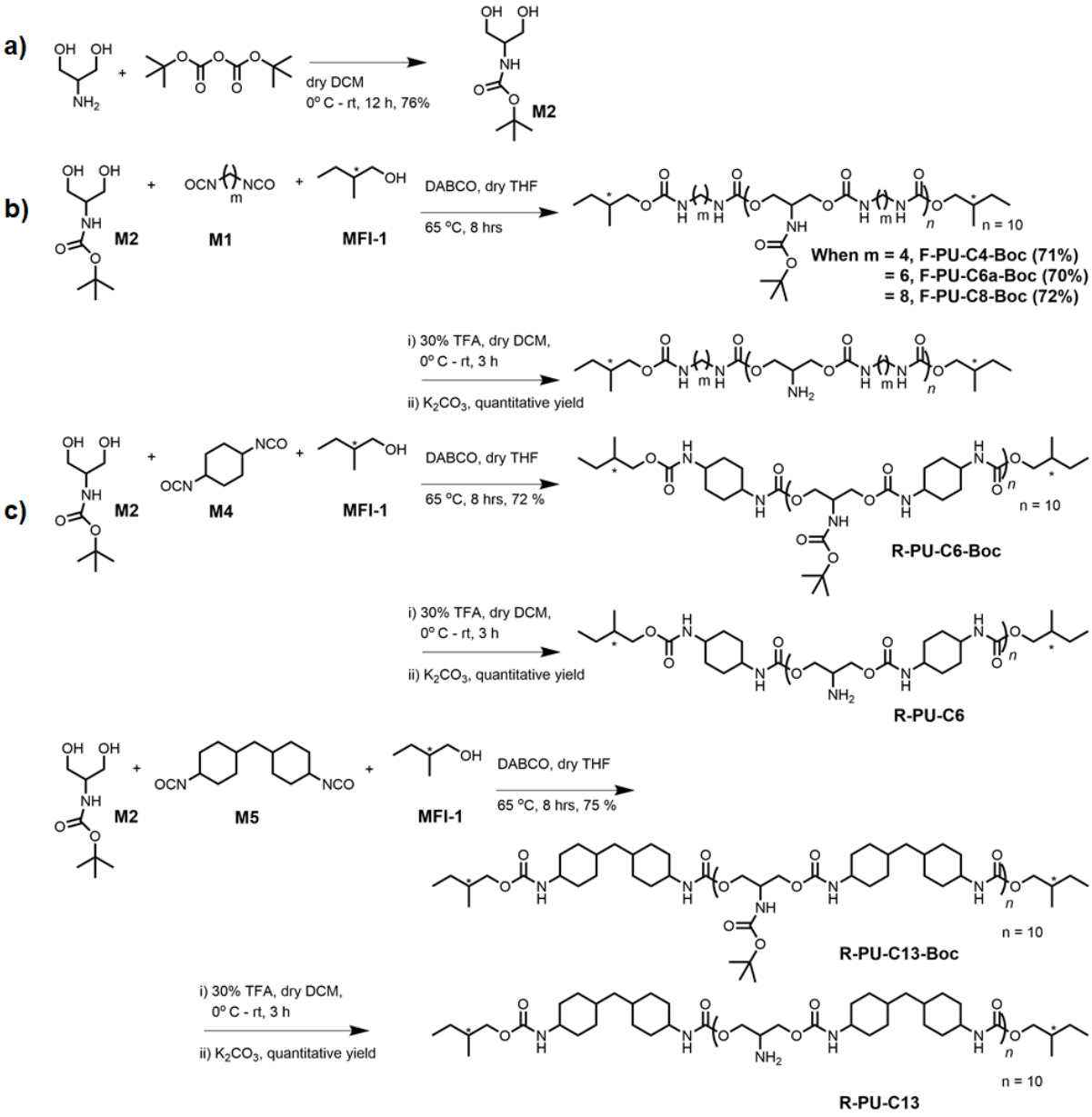
Corresponding author's email: psusg2@iacs.res.in

Materials and methods. Solvents and reagents were purchased from commercial sources and purified by reported methods.[1] HeLa cell line was obtained from NCCS, Pune, India. HEK-293 cells and all bacterial cells were purchased from ATCC, USA. DABCO was recrystalized from hexane. ^{1}H-NMR spectra were recorded from Bruker DPX-500, 400 MHz spectrometer and calibrated using TMS as the internal standard. The molecular weight of the Boc-protected polyurethanes was estimated in THF (c = 2.0 mg/ mL) at 30 °C with respect to poly(methyl methacrylate) (PMMA) standards in a Water's GPC machine equipped with a 515 HPLC pump, Waters 2414 RI detector and HSPgel HT 4.0/HSPgel HT 2.5 columns connected in series. The flow rate of the eluent was maintained at 0.6 mL/ min. Transmission Electron Microscopy (TEM) images were captured in a JEOL-2010EX machine with an accelerating voltage of 200 kV and Cryo-TEM images were captured JEOL JEM 2100 PLUS Cryo-TEM with cryogen cooled pole piece. Dynamic light scattering (DLS) and Zeta (ζ) potential measurements were performed in Malvern instrument. UV/Vis experiments were done with a JASCO V-750 spectrometer. Fluorescence experiments were carried out with a FluoroMax-3 spectrophotometer from HORIBA Jobin Yvon. X-ray diffraction (XRD) was done on a Seifert XRD3000P diffractometer with Cu Kα radiation (a = 0.154 nm) and working voltage and current of 40 kV and 30 mA, respectively. Fluorescence microscopy images were recorded in a confocal laser scanning microscopy (CLSM) in Leica TCS SP8. SEM images of bacteria

were captured in Zeiss Gemini 500 or JEOL-JSM-7500F field emission scanning electron microscopy (SEM). The absorbance of MTT and alamar blue was recorded by the microplate reader (VARIOSKAN, Thermo Fisher). CD spectra were recorded in a JASCO 1500 machine.

SYNTHESIS

Synthesis and characterization of the monomer M2, F-PU-C6a-Boc and F-PU-C6a was reported earlier by us.[2] Similar strategy was used for the synthesis of other polymers as described below.



Scheme S1. Synthesis of (a) monomer M2 (b) F-PUs and (c) R-PUs

F-PU-C4-Boc. M2 (200 mg, 1.0465 mmol) and (*S*)-(-)-2-methyl 1-butanol (MFI-1) (18.17 mg, 0.206 mmol) were taken in a glass ampule and to it 400 µL degassed anhydrous tetrahydrofuran (THF) was added and the solution was purged with dry argon gas for 10 min. Afterward, a solution of 1,4-diisocyanatobutane (M1) (160.74 mg, 1.146 mmol) in 300 µL anhydrous THF and 1,4-diazabicyclo [2.2.2] octane (DABCO) (4.69 mg, 0.041 mmol) in 100 µL anhydrous THF were added successively and the reaction mixture was further degassed for another 10 min with constant stirring. Then this homogeneous reaction mixture was heated at 65 °C for 8 h under argon atmosphere. After that the mixture became highly viscous, suggesting successful polymerization and then heating was stopped. The reaction mixture was diluted with 1.0 mL THF and the polymer was isolated as a white solid by precipitation from excess diethyl ether followed by drying under vacuum. Yield: 71 % yield. Size exclusion chromatography (SEC): M_w = 2500 g/ mol ($Đ$= 1.57). ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm):7.13 (NH urethane proton); 6.75 (NH from Boc group); 3.97 (4H, broad); 3.63 (1H, broad); 2.93 (4H, broad); 1.45 (13H, broad); 0.84 (12H, end group methyl).

F-PU-C8-Boc. F-PU-C8-Boc was synthesized by following similar method by condensation of M2 and 1,8-diisocyanato octane in presence of the MFI-1. Yield = 72 %. SEC: M_w = 4100 g/ mol ($Đ$ = 1.4). ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 7.11 (NH urethane proton); 6.75 (NH from Boc group); 3.96 (4H, broad); 3.61 (4H, broad); 2.92 (4H, broad); 1.4 (13H, broad); 1.21 (8H, broad); 0.84 (12H, end group methyl).

R-PU-C6-Boc. R-PU-C6-Boc was synthesized in similar method by condensation of M2 and *trans*-1,4-Cyclohexylene diisocyanate in presence of MFI-1. Yield = 72 %. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 7.03 (NH urethane proton); 6.94 (NH from Boc group); 3.85 (4H, broad); 3.15 (2H, broad); 1.74 (4H, Cyclohexyl ring); 1.43 (9H, broad); 1.17 (4H, cyclohexyl ring); 0.829 (12H, end group methyl).

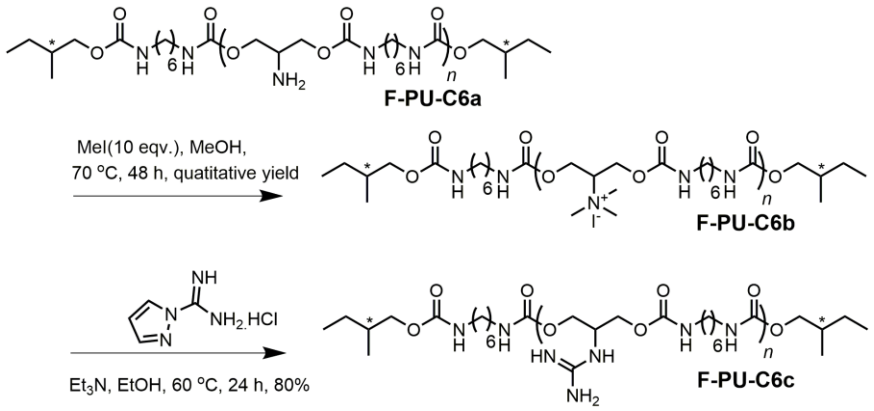
R-PU-C13-Boc. R-PU-C13-Boc was synthesized in similar method by condensation of M2 and 4,4′-Methylene bis(cyclohexyl isocyanate) in presence of the MFI-1. Yield = 75 %. SEC: M_w = 3200 g/mol ($Đ$ = 1.55). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ (ppm): 7.012 (NH urethane proton); 6.751 (NH from Boc group); 3.87 (4H, broad); 3.59 (1H, broad); 3.15 (2H, broad); 1.76-1.12 (Cyclohexyl ring); 1.43 (9H, broad); 0.84 (12H, end group methyl).

F-PU-C4. F-PU-C4-Boc (200 mg) was taken in a 25 mL round bottomed flask and dissolved in 4 mL DCM and placed in an ice bath. Then 2.4 mL TFA (30 % (v/v)) in 4 mL DCM was dropwise added to the reaction mixture and stirred for 3 h at room temperature. After that, the organic solvent was removed and fresh DCM (5 mL) was added along with 500 mg of solid K_2CO_3 and the mixture was stirred for 2 h at rt. Then the solvents were evaporated and 50 mL water was added to the crude which resulted in precipitation of the desired polymer which was centrifuged and dried under vacuum to isolated the free-amine containing polymer as a white solid in almost quantitative yield. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 7.12 (NH urethane proton); 3.83 (4H, broad); 3.04 (1H, broad); 2.958 (4H, broad); 1.37 (4H, broad); 0.86 (12H, end group methyl). Deprotection of Boc-group of rest of the polymers was done following similar procedure. FT-IR (KBR pellet, wavenumber /cm^{-1}): 3340 (N-H urethane stretching); 2927 (C-H stretching); 1700 (carbonyl stretching of urethane); 1530 (urethane N-H bending). For rest of the polymers, the characteristic peaks have also arrived at the same wavenumber.

F-PU-C8. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 7.102 (NH urethane proton); 3.844 (4H, broad); 3.008 (1H, broad); 2.955 (4H, broad); 1.48 (4H, broad); 1.22 (8H, broad); 0.86 (12H, end group methyl).

R-PU-C6. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ (ppm): 7.067 (NH urethane proton); 3.82 (4H, broad); 3.177 (2H, broad); 1.77 (4H, broad); 1.197 (4H broad); 0.85 (12H, end group methyl).

R-PU-C13. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ (ppm): 7.015 (NH urethane proton); 6.88 (NH_2 proton); 3.824 (4H, broad); 3.6 (1H, broad); 3.02 (2H, broad); 1.74-1.04 (Cyclohexyl proton, broad); 0.845 (12H, end group methyl).



Scheme S2. Synthesis of F-PU-C6b and F-PU-C6c.

F-PU-C6b. To a 15 mL reaction shield tube, polymer F-PU-C6a (100 mg, 0.386 mmol) was dissolved in 5 mL MeOH. Then, Methyl iodide (240 µL, 3.86 mmol) was added to the reaction mixture and heated at 70 ^{o}C for 48 h under shield condition. Then reaction mixture was allowed to cooled at room temperature and the polymer was precipitated from diethyl ether. The polymer was purified by dialysis against MeOH water mixture for 48 h and then solid yellow coloured polymer was collected by lyophilization with quantitative yield. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 7.08 (NH urethane proton); 3.98 (1H, broad); 3.89 (4H, broad); 3.21 (s, 9H); 2.9 (4H, broad); 1.35 (4H, broad) 1.21 (4H, broad); 0.84 (12H, end group methyl).

F-PU-C6c. A post-polymerization guanylation method was followed to convert primary amine groups of polymers F-PU-C6a to give guanidine functionalized polymer F-PU-C6c. In a 25 mL round bottom flask polymer F-PU-C6a (100 mg, 0.386 mmol) was dissolved in anhydrous ethanol. Then 1H-pyrazole-1-carboxamidine hydrochloride (169 mg, 1.15 mmol) and triethyl amine (310 µL, 2.31 mmol) were added to the reaction mixture and heated at 60 ^{o}C under nitrogen for 24 h. Then the polymer was purified by dialysis against water and light yellow coloured solid polymer was collected by lyophilization with 80% yield. The polymer was

characterization by ^{1}H NMR spectra. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 7.08 (NH urethane proton); 3.93 (1H, broad); 3.89 (4H, broad); 2.9 (4H, broad); 1.35 (4H, broad) 1.21 (4H, broad); 0.84 (12H, end group methyl).

EXPERIMENTAL DETAIL ON SELF-ASSEMBLY STUDIES

HRTEM, Cryo-TEM, DLS and ζ-potential measurements. A measured amount of a given polymer was taken in a glass vial along measured volume of 2.0 mM aqueous HCl solution so that 1.0 mole equivalent of HCl is present in the mixture with respect to its repeat unit molecular weight of the polymer. The mixture was sonicated for 2-min and then to it measured volume of distilled water was added so that the concentration of the polymer became 1.0 mM (based on the repeat unit molecular weight). The solution was subjected to dialysis for 24 h (dialysis bag MWCO = 3000 Da) against distilled water to remove any unreacted acid. For F-PU-C6b, the measured polymer was dissolved in distilled water without HCl solution to prepare 1.0 mM stock solution. For HRTEM, the polymer solution was drop-cast on a carbon-coated Cu grid and air-dried for 24 h before imaging. For cryo TEM images, one drop of the aqueous solution of F-PU-C6b (c = 1 mg/ mL) was syringed out and mounted on 300 mesh carbon coated Cu grid hung on to GATAN cryo plunger which was then immediately transferred to a cryogen cooled specially designed GATAN sample holder and examined under 120 kV electron beam. For DLS and ζ-potential measurements, the concentration was adjusted to 100 µg/ mL.

Calcein and Rhodamine-B encapsulation study. 30 µL of Calcein stock solution (10^{-3} M) in MeOH was mixed with 100 µL of polymer solution (30 mM) and to this 2.87 mL of acidic water (1.0 equiv. HCl w.r.t. polymer concentration) was dropwise added so that the final polymer concentration was 1.0 mM. Then, the mixture was subjected to dialysis (MWCO = 3000 Da) for 48 h to remove MeOH or any non-encapsulated dye. Then the volume was adjusted to 3.0 mL by adding a measured amount of water to this solution. Similar procedure was followed for Rhodamine-B encapsulation. After that, UV/vis spectra of the solutions were

checked, which showed a peak with λ_{max} = 490 nm and 554 nm, respectively, for the Calcein and Rhodamine-B treated samples, indicating successful encapsulation. Emission spectra of the solutions were recorded and compared with corresponding absorption-matched emission spectra of the free dye in water. To determine the critical aggregation concentration, emission intensity of Calcein encapsulated F-PU-C6a solution was checked in different concentration by serial half-dilution of the 1.0 mM stock solution.

Powder X-ray Diffraction Studies. Aqueous solution of each polymer (10 mg/ mL) was drop-casted repeatedly on a cleaned glass slide to make a thick film, and then the film was air-dried for 24 h. Data were recorded with this sample from 0.5° to 30° with data interval of 0.02 Å per state.

Small-angle neutron scattering (SANS). Small-angle neutron scattering experiments were performed at the SANS diffractometer at Guide Tube Laboratory, Dhruva Reactor, Bhabha Atomic Research Centre, Mumbai, India.[3] In SANS, one measures the coherent differential scattering cross-section ($d\Sigma/d\Omega$) per unit volume as a function of wave vector transfer Q ($= 4\pi \sin\theta/\lambda$, where λ is the wavelength of the incident neutrons and 2θ is the scattering angle). The mean wavelength of the monochromatized beam from neutron velocity selector is 5.2 Å with a spread of $\Delta\lambda/\lambda \sim 15\%$. The angular distribution of neutrons scattered by the sample is recorded using a 1.0 m long one-dimensional He^3 position sensitive detector. The instrument covers a Q-range of 0.015-0.35 $Å^{-1}$. The data have been analyzed by comparing the scattering from different models to the experimental data. The modelling of the SANS data is described in detail in the next section.

Small-angle neutron scattering data analysis. The differential scattering cross-section per unit volume ($d\Sigma/d\Omega$) as measured for a system of monodisperse particles in a medium can be expressed as [4-5]

$$\left(\frac{d\Sigma}{d\Omega}\right)(Q) = nV^2\left(\rho_p - \rho_s\right)^2 P(Q)S(Q) + B \ , \tag{1}$$

where n denotes the number density of particles, ρ_p and ρ_s are respectively, the scattering length densities of particle and solvent and V is the volume of the particle. $P(Q)$ is the intraparticle structure factor and $S(Q)$ is the interparticle structure factor. B is a constant term representing incoherent background, which is mainly due to the hydrogen present in the sample.

Intraparticle structure factor $P(Q)$ is decided by the shape and size of the particle and is the square of single-particle form factor $F(Q)$ as determined by

$$P(Q) = \langle |F(Q)|^2 \rangle \ . \tag{2}$$

For a spherical particle of radius R, $F(Q)$ is given by [6]

$$F(Q) = 3\left[\frac{\sin(QR) - QR\cos(QR)}{(QR)^3}\right] . \tag{3}$$

For a system of monodisperse unilamellar vesicles, $d\Sigma/d\Omega$ can be expressed as [7]

$$\frac{d\Sigma}{d\Omega}(Q,R) = n(\rho_v - \rho_s)^2\left[\frac{4}{3}\pi R^3\frac{3J_1(QR)}{QR} - \frac{4}{3}\pi(R+t)^3\frac{3J_1\left[Q(R+t)\right]}{Q(R+t)}\right]^2 \tag{4}$$

where n denotes the number density of the vesicles, ρ_v and ρ_s are the scattering length densities of the vesicle bilayer and the solvent, respectively. R is the radius of the vesicle and t is the thickness of the bilayer. $J_1(x)$ is the first order Bessel function and is given by

$$J_1(x) = \frac{\sin x - x\cos x}{x^2} \tag{5}$$

The polydispersity in size distribution of particle is incorporated using the following integration[8]

$$\frac{d\Sigma}{d\Omega}(Q) = \int \frac{d\Sigma}{d\Omega}(Q,R)\, f(R)dR + B \ , \tag{6}$$

where $f(R)$ is the size distribution of the vesicles and usually accounted by a log-normal distribution as given by

$$f(R) = \frac{1}{\sqrt{2\pi}R\sigma} \exp\left[-\frac{1}{2\sigma^2} \left(\ln \frac{R}{R_{med}} \right)^2 \right], \quad (7)$$

where R_{med} is the median value and σ is the standard deviation (polydispersity) of the distribution. The mean radius (R_m) is given by $R_m = R_{med} \exp(\sigma^2/2)$.

The data have been analysed by comparing the scattering from different models to the experimental data. Throughout the data analysis, corrections were made for instrumental smearing, where the calculated scattering profiles smeared by the appropriate resolution function to compare with the measured data.[9] The fitted parameters in the analysis were optimized using nonlinear least-square fitting program to the model scattering.

ANTIBACTERIAL STUDIES

Bacterial preparation. All the bacteria were grown aerobically in Luria-bertani broth/ agar at 37 ^{o}C with gentle shaking at 180 rpm and bacteria of mid-logarithmic phase was used for antibacterial study. Gram-positive *S. aureus* (ATCC 25923) and Gram-negative bacteria *E. coli* (ATCC 25922), *E. coli* TOP10 (ATCC PTA-10989) and *E. coli* DH5α (ATCC 68233) were used for minimum inhibitory concentration (MIC) determination.

Minimum Inhibitory Concentration (MIC) determination. The MIC value of a given sample solution was determined by a broth microdilution assay. Serial dilutions were done with a 1.0 mg/ mL concentrated stock polymer solution to get different concentrations of the sample in LB broth. Then 50 μL of bacterial solutions (c = 1× 10^6 CFU/ mL) were added to each well of 96-well cell culture plate (round bottom with polystyrene lid, non-pyrogenic, polypropylene, corning). Then it was incubated at 37 °C for 18 h with gentle shaking at 180 rpm. The MIC was considered as the lowest concentration of polymer solution at which there was 100%

reduction in growth. Broth alone and broth containing only cells were also incubated as sterility control and growth control, respectively. All experiments were done in triplicate.

Minimum Bactericidal Concentration (MBC) determination: Different concentration of F-PU-C6c solution was prepared in LB media and the bacteria (c = 5×10^5 CFU/ mL) solution was incubated with these polymer solutions for 18 h similar to what was done for the MIC determination. Then a loopful of cultured broth from each well was inoculated in a luria bertani agar plate. The plate was incubated at 37 °C for 24 h. After 24 h, MBC was determined by inspection of agar plate. Concentration at which no growth of the bacterial colony was observed was taken as the MBC.

Hemolysis Assay. The hemolysis study was performed in compliance with the guidelines of the Indian Council of Medical Research (ICMR) which was approved by the Institutional Ethics Committee of IICB, Kolkata, India. After obtaining informed consent, human RBCs from a healthy voluntary donor (1.0 mL) were added in 9.0 mL of freshly prepared PBS buffer (pH 7.4) and centrifuged at 2000 rpm for 5 min. The supernatant was removed and resuspended in the same PBS. This washing was done for two additional times. Finally, the RBC pellet was suspended in 10 mL of volume and then 2.5% (v/v) RBC solution was prepared by four times dilution of the stock solution. Then, different concentrated polymer solution was prepared by serial dilution in PBS in a sterile 96-well flat-bottom polystyrene plate and 150 μL of RBC suspension 2.5 % (v/v) was added to each well and incubated at 37 °C with shaking at 180 rpm for 1 h. Triton X-100 (0.1% v/v in water) and PBS solution were used as the positive lysis control and negative control respectively. Supernatant (100 μL) from each well was transferred to a sterile 96-well flat-bottom polystyrene plate and the absorbance was measured at 414 nm using a Varioskan microplate reader (Thermo Fisher). The percentage of hemolysis was calculated relative to the positive control and negative control solvents.

Killing kinetic assay. Killing curve was made to determine the different bactericidal activity among the polymers for *E. coli* (ATCC 25922). 500.0 μL of polymer solution was mixed with bacterial solution and incubated at 37 °C with gentle shaking at 180 rpm where final concentration of polymer and bacteria are 156 μg/ mL and 5×10^5 CFU/ mL respectively. At different time intervals, 100 μL of the solution was taken, diluted 100 times, and plated on LA plate followed by overnight incubation at 37 °C. The next day, the colony-forming units (CFU) were counted. The assay was repeated three times in each case.

Cytotoxicity assay using MTT. Hela cells or HEK-293 (approximately 10000 cells/ well) were seeded in 96-well plates and after 24 h of incubation, the medium was replaced by the different concentrated polymer solution and incubated for 24 and 48 h. Then, 50 μL of freshly prepared 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (c = 5 mg/ mL in DMEM) was added into each well and incubated for 4 h. After that the medium with MTT solution was carefully removed and 100 μL of DMSO was added into each well, and the plate was gently shaken for 5 min at room temperature to dissolve all formed precipitates. The absorbance at 570 nm was recorded by a microplate reader (VARIOSKAN, Thermo Fisher). Cell viability was calculated by the ratio of absolute absorbance of the cells incubated with the polymer suspension and the cells incubated with only the culture medium. Experiments were repeated three times and data reported with standard deviation based on data obtained from three independent experiments.

Scanning Electron Microscopy (SEM). 500 μL of *E. coli* (ATCC 25922) or *S. aureus* (ATCC 25923) suspensions (1×10^9 CFU/ mL) in LB broth were mixed with 500 μL of polymer solution (200 μg/ mL) and the resulting solution was incubated for 4 h at 37 °C. After that the suspensions were centrifuged at 4000 rpm for 10 min and precipitates were washed with 0.85% NaCl solution twice and then it was resuspended in 2.5% glutaraldehyde in PBS, incubated at room temperature for 30 min and overnight at 4 °C. Then, it was centrifuged to collect the

pellets and washed with DI water twice. After that the samples were dehydrated in different ethanol grades (10, 30, 50, 70, 80, 90, and 100%, 15 min each step). Samples were spread on glass plates and 1.5 min platinum spattering was done before capturing the images. Control and F-PU-C6a treated *S. aureus* were imaged using Zeiss Gemini 500 Field Emission Scanning Electron Microscope and other bacterial images were captured using a JEOL-JSM-7500F field-emission scanning electron microscope at 5.0 kV

Cytoplasmic membrane permeability assays. *E. coli* (ATCC 25922) was grown in modified Luria-Bertani broth with 2% lactose. Then, the cell suspension was centrifuged at 2000 rpm for 10 min and cell pellets were washed and resuspended in PBS (pH = 7.4). Next, the *E. coli* suspension (OD_{600} = 0.1) was mixed in a 1:1 ratio with polymer solutions and then ONPG solution in PBS was added to it where the final concentration of the polymer and ONPG are 125 μg/ mL and 1.5 mM respectively. Then, 1.0 mL of the solution was taken in a cuvette (pathlength = 1.0 cm) and then the absorbance at 420 nm was measured in every 2.0 min for 100 min at 25 ^{o}C using a JASCO V- 750 spectrophotometer. Controlled experiments were performed following the same conditions in the absence of any polymer solution.

Membrane depolarization. Cytoplasmic membrane depolarization by polymer solution was checked using a potential sensitive dye 3,3′-dipropylthiacarbocyanine ($diSC_3(5)$. A solution of *E. coli* bacteria was prepared in a buffer composing with 5 mM HEPES, 20 mM glucose, and 100 mM KCl, pH 7.2. Then to a 1.0 mL of bacterial solution was mixed with 5 μL of 10 μM $diSC_3(5)$ solution where the final concentration of cells and dye are 5×10^7 CFU/ mL and 50 nM respectively. The fluorescence intensity of the dye was monitored in each 1.0 min time intervals for 17 min at 25 °C at an excitation wavelength of 622 nm and an emission wavelength of 670 nm. Fluorescence intensity of the dye starts to decrease with time as dye localised in membrane resulting the self-quenching. After 5 min, the dye uptake in the membrane reached to its maximum and then 10.0 μL of 2 mg/ mL concentrated polymer solution (final

concentration = 100 µg/ mL) was added to the 200 µL of cell suspension in the cuvette. The fluorescence intensity increased gradually as the membrane potential decreases due to the interaction of membrane with the polymer aggregates which resulted the release of the dye from the cell membrane. Measurements were repeated at least twice under the same condition.

Biofilm susceptibility. Biofilm susceptibility was assessed using alamar blue dye as a marker which reduced by metabolically active cells from indigo blue to fluorescent pink. Firstly, 100 µL of cells (1×10^7 CFU/ mL) was seeded in each well of flat-bottomed 96-well plate and incubated at 37 ^oC for 4 h in static condition to adhere the cells on the bottom surface in each well. Then the supernatant with non-adherent cells was removed carefully and washed twice with 100 µL of 0.85 % NaCl (w/v) solution. Then 100 µL fresh medium was added to each well and further incubated for 24 h. After that the supernatant was removed and washed with 100 µL of 0.85 % NaCl solution. Next, 100 µL of different concentrated polymer solution in LB media was added to each well and control was made by adding 100 µL of only medium without any polymer solution. The plate was incubated for 3 h at 37 ^oC in static condition and then the supernatant was removed and washed with 0.85 % NaCl solution. Next, 100 µL of alamar blue solution was added to each well and incubated in dark for 5-7 h. The alamar blue solution was prepared in 0.85 % NaCl by ten times dilution of commercially available alamar blue solution (purchased from thermo fisher). The fluorescence intensity was recorded at an excitation wavelength of 570 nm (λ_{ex} = 570 nm) and an emission wavelength of 590 nm (λ_{em} = 590 nm) and excitation band width of 5 nm using a microplate reader. The percentage of biofilm killing (% of B.K) was estimated by the following formula:

% of B. K = [(Fluorescence intensity of control - Fluorescence intensity of tested well) / Fluorescence intensity of control] ×100.

Confocal microscopy of biofilm: This biofilm viability was visualized by confocal microscopy using the Live/Dead *Bac* Light^{TM} kit (Invitrogen). First bacterial solution (1×10^7

CFU/ mL) in LB media was seeded on confocal disc and incubated for 4 h in the static condition at 37 ^{o}C to adhere on the surface and next supernatant was carefully removed and 100 µL fresh LB media was added and incubated for another 24 h. Next, the supernatant was again removed and 100 µL of polymer solution (c = 125 µg/ mL) was added and incubated for 3 h and then supernatant was removed and then 100 µL of staining solution (1.5 µL of syto-9 and 1.5 µL propidium iodide was mixed in 100 µL 0.85% NaCl solution) was added and incubated for 15 min in the dark. Then the solution was removed and plate was washed with 0.85 % NaCl solution to remove the extra dye. The biofilm viability was checked by confocal laser scanning microscopy (CLSM) in Leica TCS SP8.

TABLE AND FIGURES

Table S1. Structural characterization the polymers

Polymer	(X_n) (NMR)	X_n (theoretical)[a]	M_n (theoretical)[a]	Mol. Wt. (NMR)	M_n (SEC)	$Đ$
F-PU- C4-Boc	22	19	3464	3640	2500	1.57
F-PU- C6a-Boc	20	19	3750	4300	3900	1.80
F-PU- C8-Boc	20	19	4053	4260	4100	1.40
R-PU- C6-Boc	20	19	3735	3920	-[b]	-
R-PU-C13-Boc	20	19	4744	4530	3200	1.55

[a] X_n (Number average degree of polymerization) was estimated from the Carothers equation wherein the monomer ratio $r = N_{AA} / (N_{BB} + N_{A*})$ and conversion ρ was considered to be 0.99. M_n (theoretical) = (X_n/2 X mass of the repeating unit) + mass of the end groups; [b] This polymer was not soluble in THF and thus SEC experiment could not be conducted. Although synthesis of F-PU-C6a-Boc was reported earlier by us[2], data presented in this table for comparison purpose.

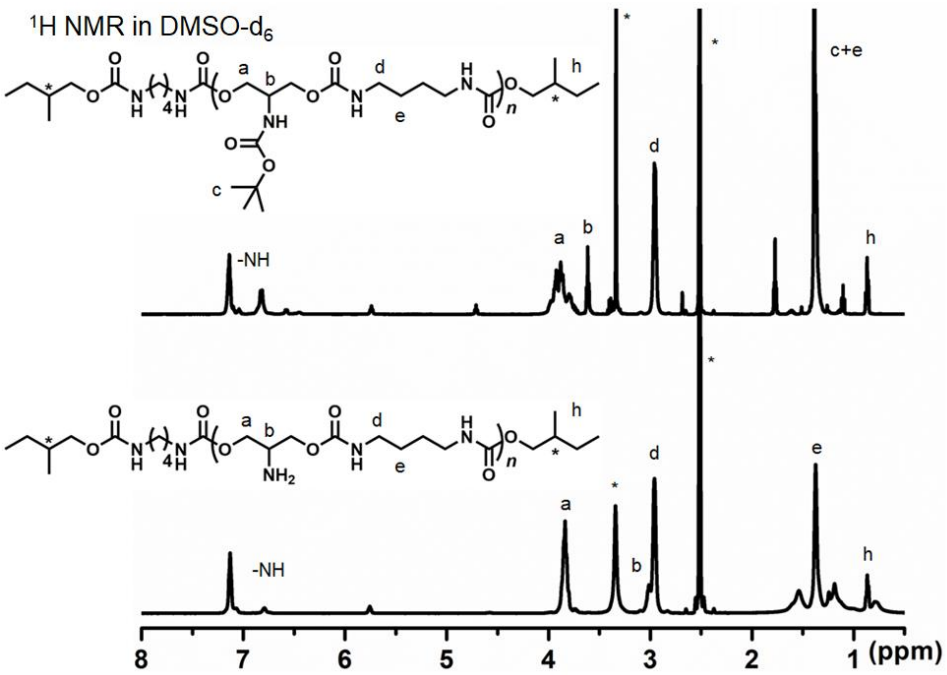


Figure S1a. ^{1}H-NMR spectra of F-PU-C4-Boc and F-PU-C4 in DMSO-d_6 (* indicates peaks from residual solvent).

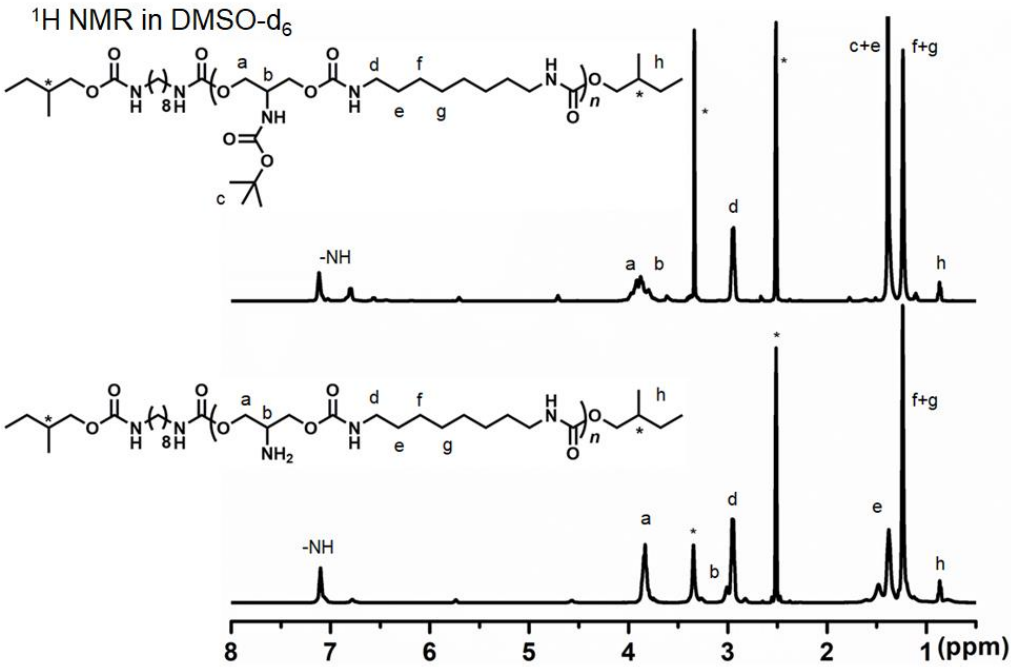


Figure S1b. ^{1}H-NMR spectra of F-PU-C8-Boc and F-PU-C8 in DMSO-d_6. (*indicates residual peaks from the solvent)

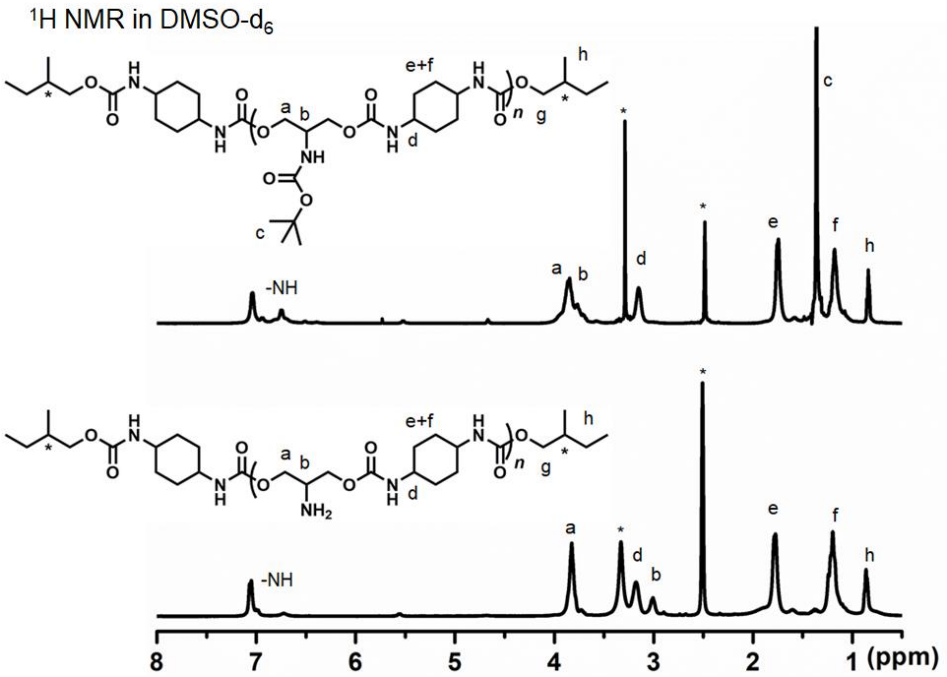


Figure S1c. ^{1}H-NMR spectra of R-PU-C6-Boc and R-PU-C6 in DMSO-d_6 (*indicates residual peaks from the solvent).

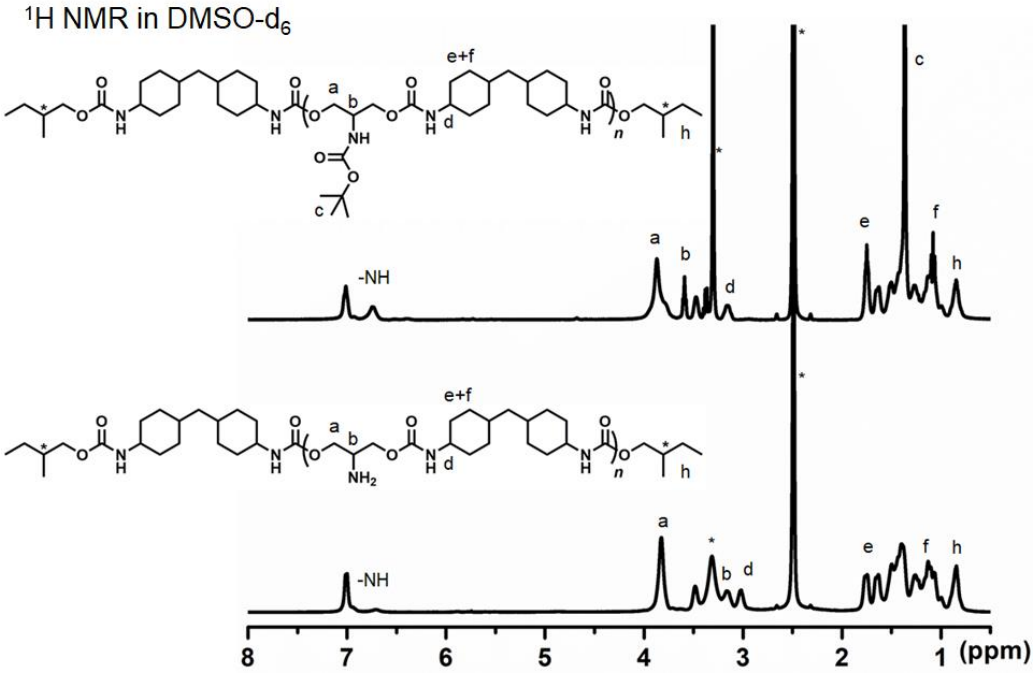


Figure S1d. ^{1}H-NMR spectra of R-PU-C13-Boc and R-PU-C13 in DMSO-d_6 (*indicates residual peaks from the solvent).

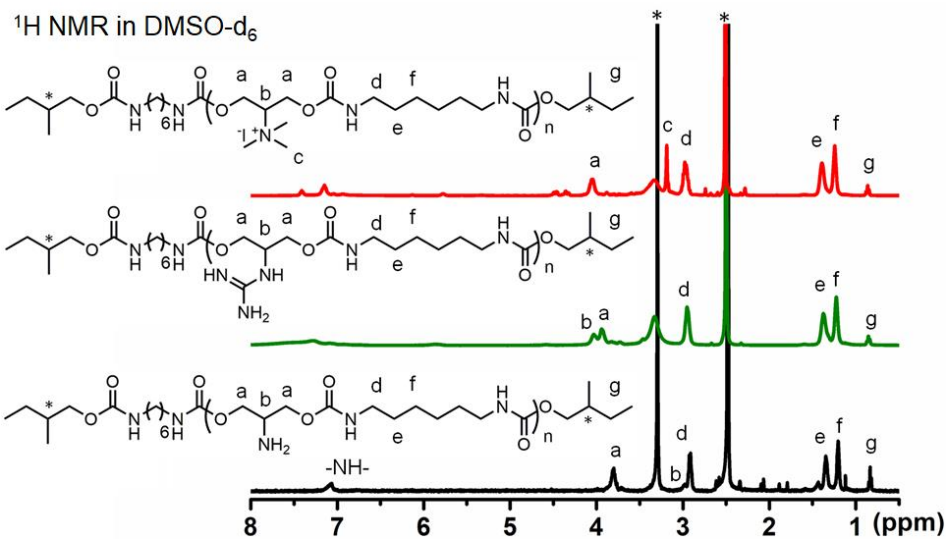


Figure S1e. ^{1}H-NMR spectra of F-PU-C6a, F-PU-C6b and F-PU-C6c in DMSO-d_6 (*indicates residual peaks from the solvent). ^{1}H-NMR of F-PU-C6a was reported earlier by us[2], same data presented in this figure together with new polymers for comparison purpose.

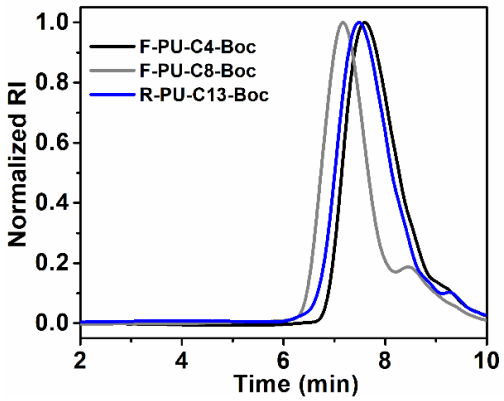


Figure S2. SEC traces of Boc-protected newly synthesized polymers in THF.

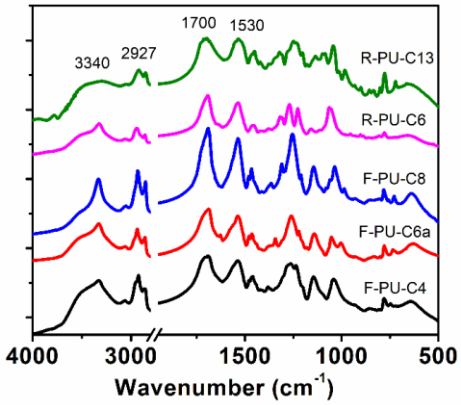


Figure S3. Solid state FTIR spectra of the polymers. Peaks at 3340 cm^{-1}, 2927 cm^{-1}, 1700 cm^{-1}, and 1530 cm^{-1} are assigned to the N-H urethane stretching, C-H stretching, urethane carbonyl stretching and urethane N-H bending, respectively

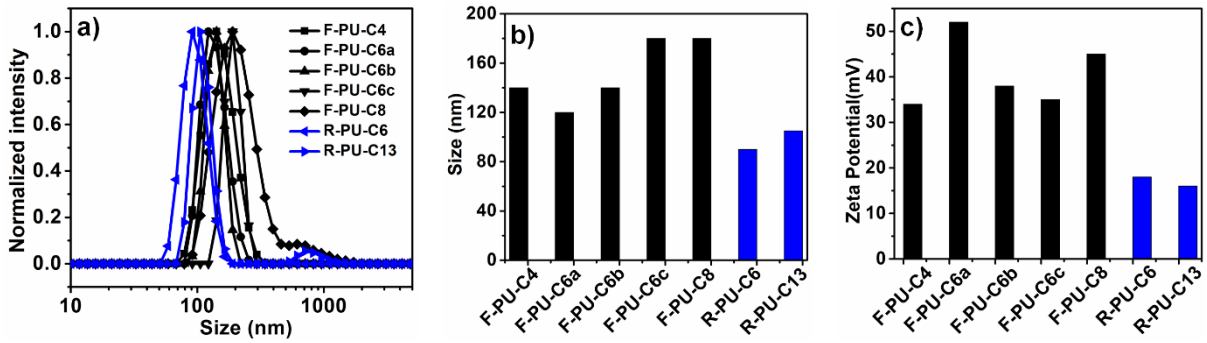


Figure S4. a) DLS profile and variation in b) D_h and c) zeta potential of aqueous aggregates of the polyurethanes (c = 100 µg/ mL, pH 6). Although DLS of F-PU-C6a was reported earlier by us[2], same data presented in this figure together with new polymers for comparison purpose.

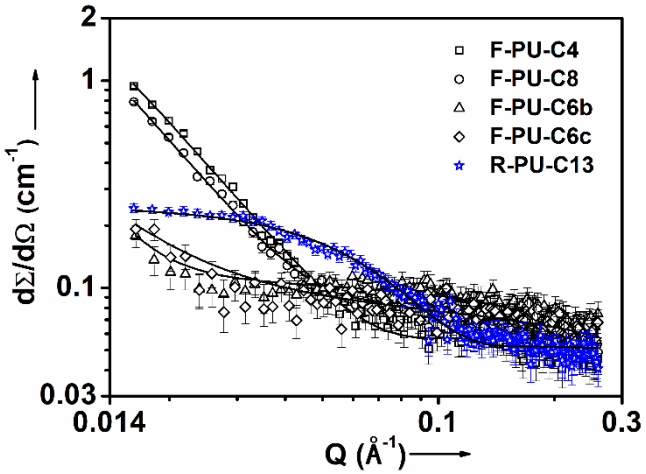


Figure S5. Plot of scattering intensity as a function of scattering vector *Q*.

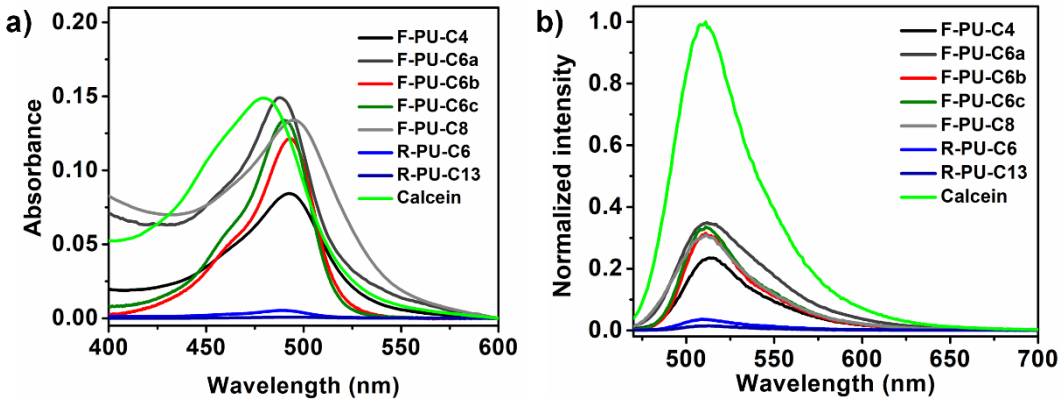


Figure S6. (a) UV-Vis spectra and (b) Normalized emission spectra (λ_{ex} = 450 nm) of Calcein-treated polymers and the free dye. Concentration of polymer = 1.0 mM. Concentration of Calcein varies as indicated by the different intensity in the absorption spectra. Almost no encapsulation in evident for R-PU-C6 or R-PU-C13 from the absorption spectra. For F-PUs although the absorption intensity is comparable to the free dye, the reduced emission intensity

indicates self-quenching in the confined environment of the polymer aggregates. For R-PUs, negligible emission intensity indicates negligible encapsulation. For F-PU-C6a encapsulated sample and only Calcein same data those were reported before [2] have been used for comparison purpose.

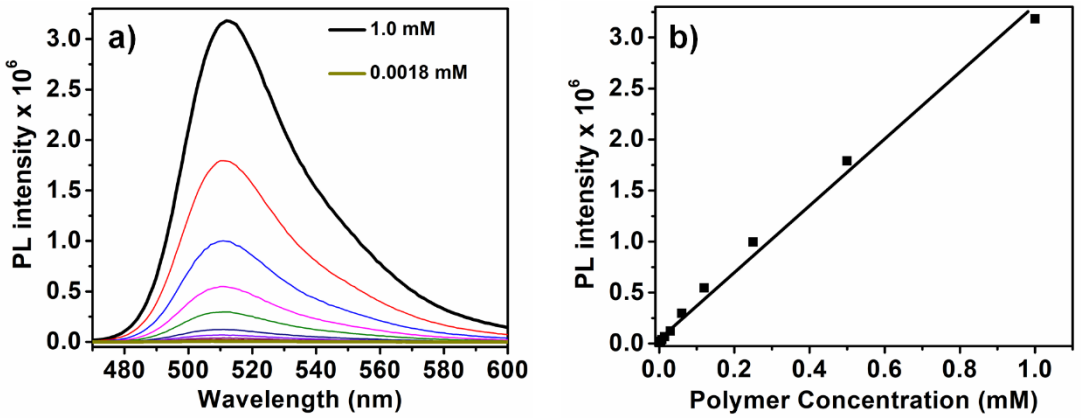


Figure S7. a) Concentration dependent (1.0 mM to 0.0018 mM) emission spectra of Calcein encapsulated aqueous aggregate of F-PU-C6a (λ_{ex} = 450 nm); d) Variation of maximum fluorescence intensity (509 nm) with polymer concentration.

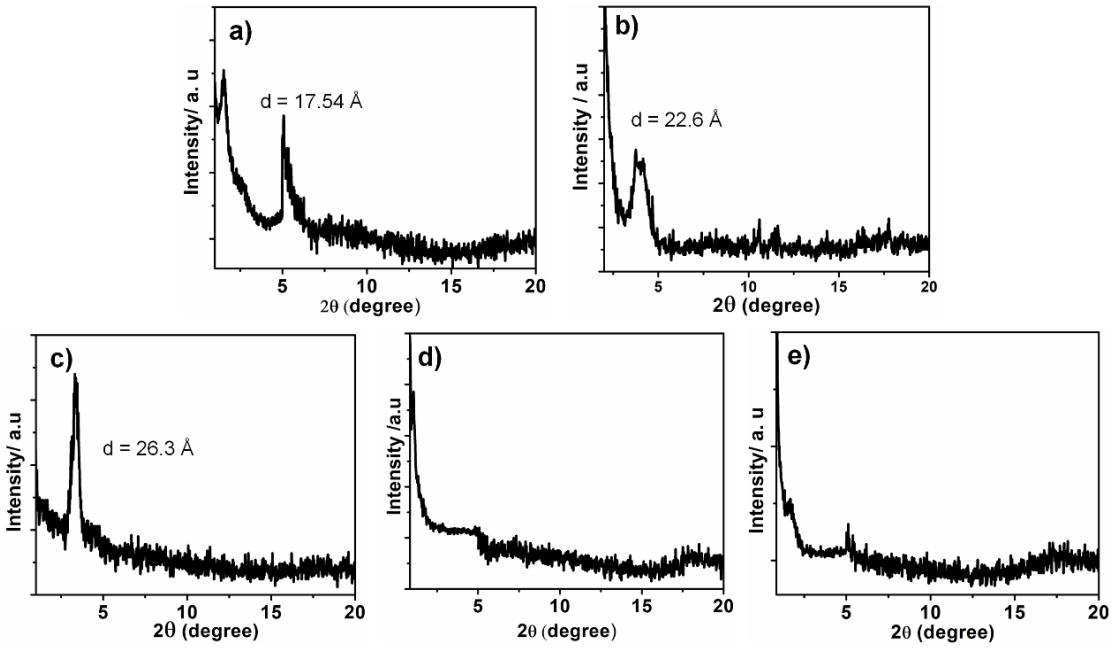


Figure S8. (a) XRD pattern of the dried film produced from aqueous solution of (a) F-PU-C4, (b) F-PU-C6a, (c) F-PU-C8, (d) R-PU-C6 and (e) R-PU-C13 (*c* =10 mg/ mL). For F-PUs, sharp

peak at the small angle indicates well-defined plane by pleated conformation of the polymer chain which is missing for the R-PUs.

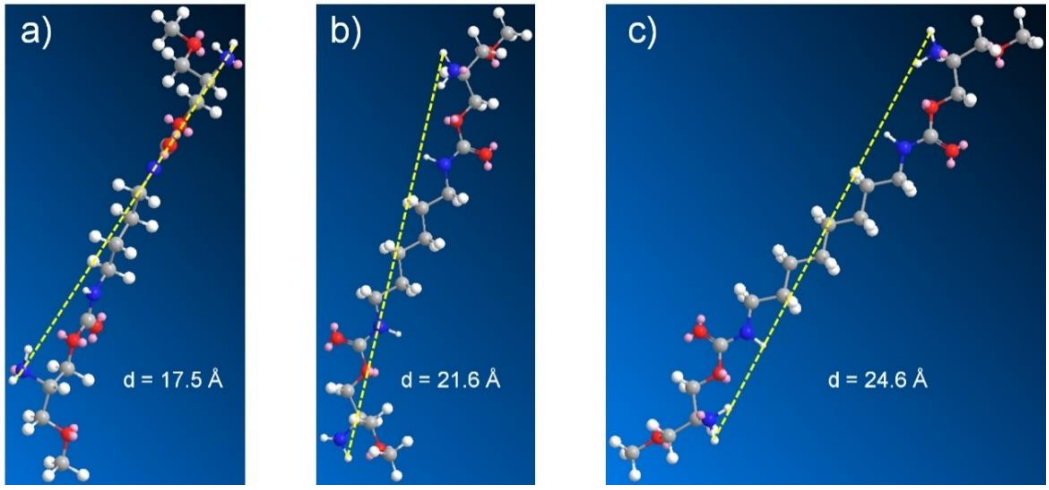


Figure S9. Energy minimized structure of (a) F-PU-C4, (b) F-PU-C6a and (c) F-PU-C8 by molecular modelling done in Chem 3D 18.0 using MM2 for energy minimization.

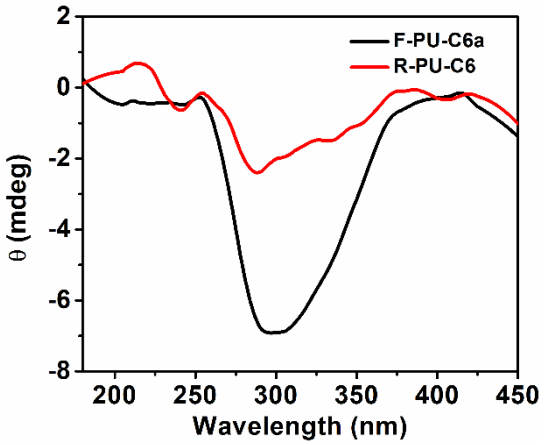


Figure S10. CD spectra of F-PU-C6a and R-PU-C6 (*c* = 0.5 mg/ mL).

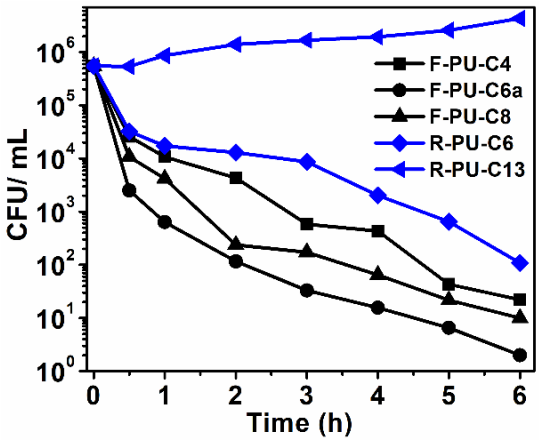


Figure S11. Bactericidal kinetics of the polymers against *E. coli* (ATCC 25922).

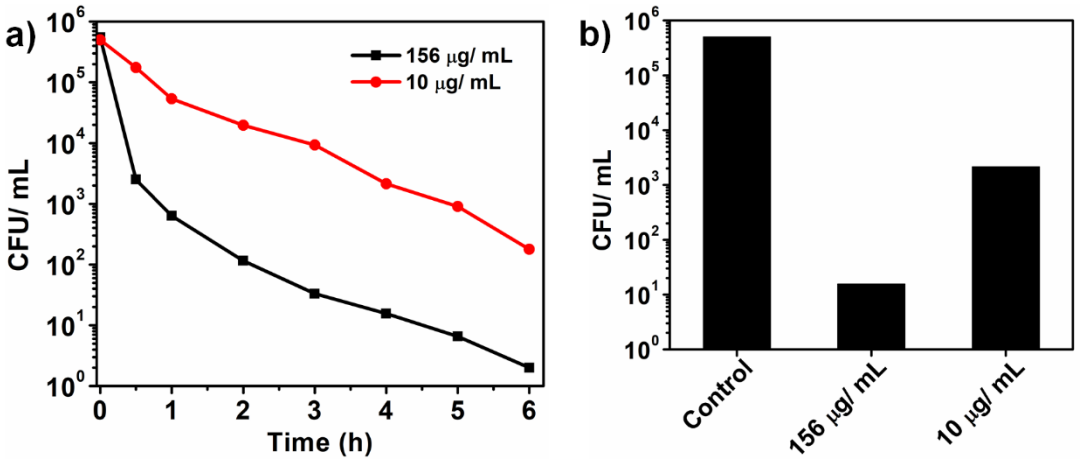


Figure S12. a) Bactericidal killing kinetics of F-PU-C6a against *E. coli* (ATCC 25922) at different concentration and b) Reduction of viable bacteria after 4 h of incubation.

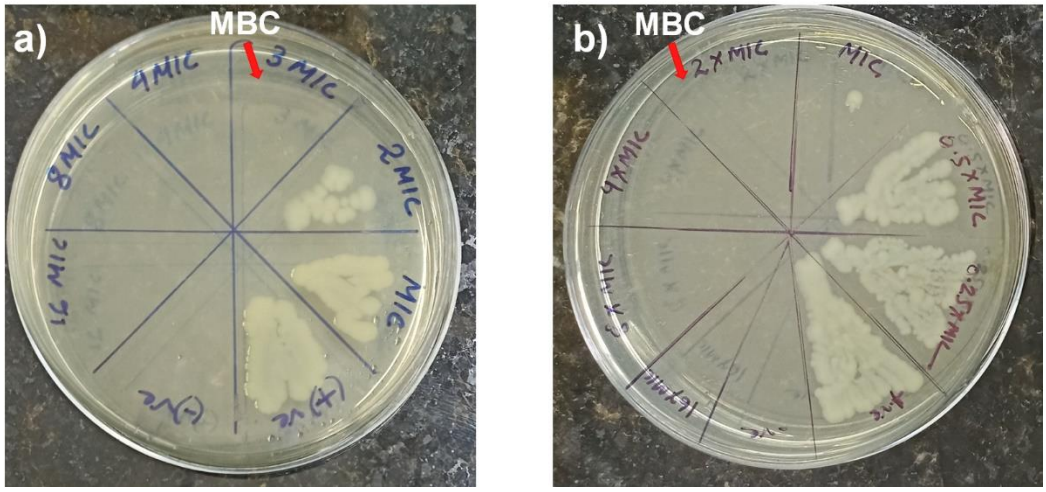


Figure S13. Image of LB agar plate used for determination of minimum bactericidal concentration (MBC) of F-PU-C6c for a) *E. coli* (ATCC 25922) and b) *S. aureus* (ATCC 25923). The concentration of polymer was considered as MBC where complete eradication of bacteria colony was observed (indicated by red arrow). MBC for *E. coli* = 8.1 µg/ mL (3 X MIC) and MBC for *S. aureus* = 8.0 µg/ mL. (2 X MIC). MIC values are reported in Table 2. =

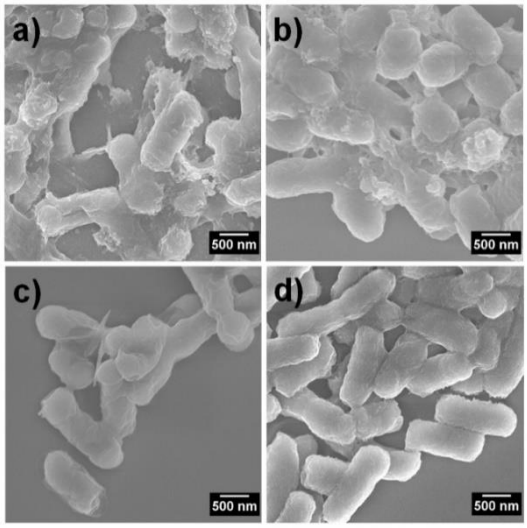


Figure S14. SEM images of *E. coli* bacteria (ATCC 25922) after 4 h of incubation with a) F-PU-C4, b) F-PU-C8, c) R-PU-C6 and d) R-PU-C13. *c* = 100 µg/ mL.

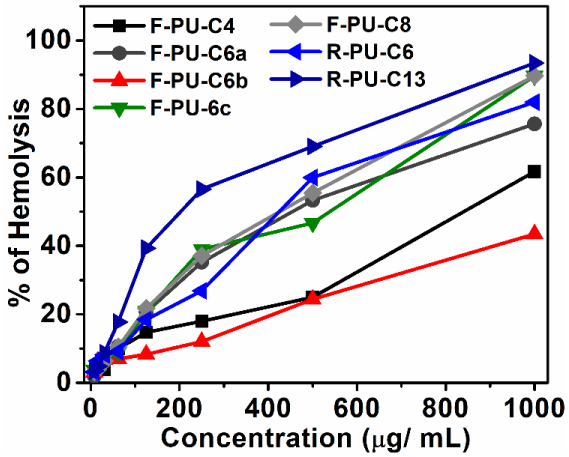


Figure S15. Hemolysis activity of the polymers.

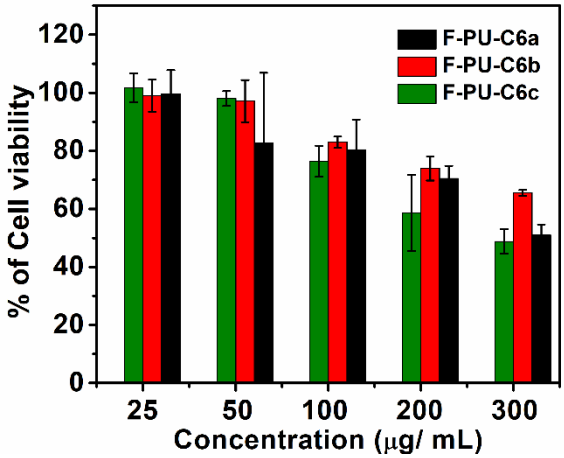


Figure S16. Comparison of the cell viability study results of highly bactericidal F-PU-C6a, F-PU-C6b, F-PU-C6c using HEK-293 cells after 24 h of incubation.

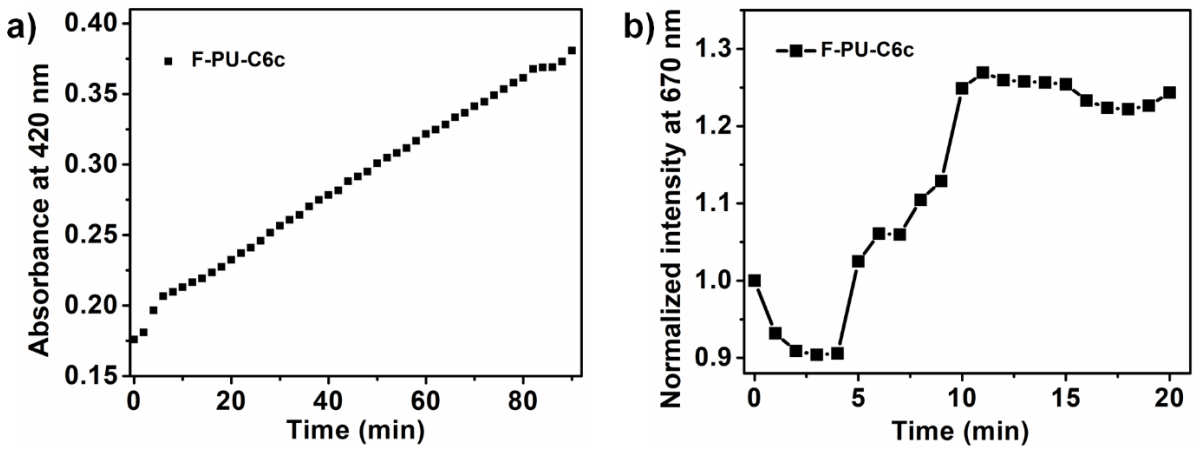


Figure S17. a) Membrane disruption result by ONPG assay and b) Cytoplasmic membrane depolarization assay result using membrane sensitive dye $diSC_3(5)$ of *E. coli* (ATCC 25922) by F-PU-C6c (*c* = 5 µg/ mL).

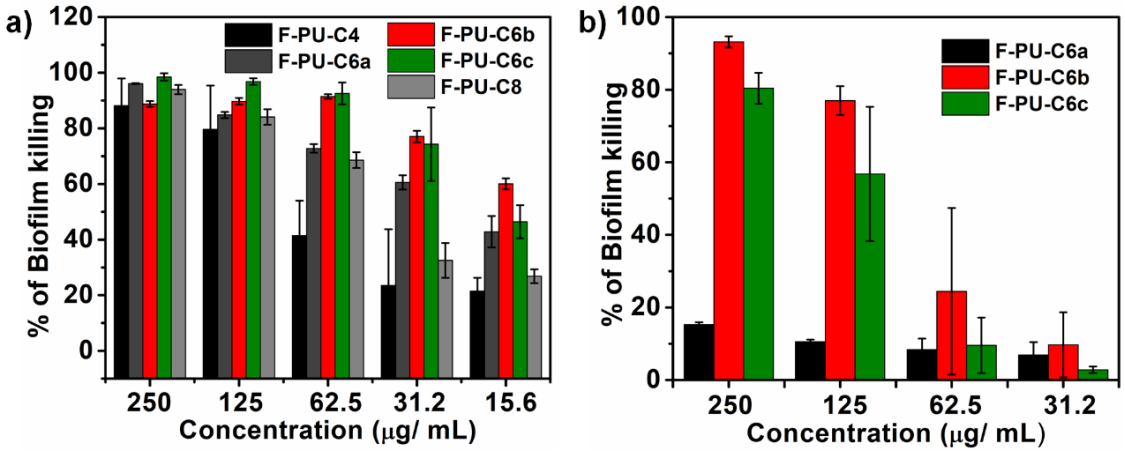


Figure S18. Biofilm killing study of a) F-PUs using the biofilm prepared by *E. coli* (ATCC 25922) and b) F-PU-C6a, PU-C6b, F-PU-C6c using the biofilm of *S. aureus* (ATCC 25923). Polymers were treated for 3 h on biofilm prepared by 3-days of ageing.

REFERENCES

1. D. D. Perrin, W. L. F. Armarego and D. R. Perrin, *Purification of Laboratory Chemicals*, Pergamon Press: **1986**
2. R. Barman, T. Mondal, J. Sarkar, A. Sikder and S. Ghosh, *ACS Biomater. Sci. Eng.*, 2020, **6**, 654.
3. V. K. Aswal and P.S. Goyal, *Curr. Sci.*, 2000, **79**, 947.
4. J. B. Hayter and J. Penfold, *Colloid Polym. Sci.*, 1983, **261**, 1022.

5. E. W. Kaler, *J. Appl. Cryst.*, 1988, **21**, 729.
6. J. B. Hayter and J. Penfold, *Mol. Phys.*, 1981, **42**, 109.
7. P. Pramanik, D. Ray, V.K. Aswal and S. Ghosh, *Angew. Chem., Int. Ed.*, 2017, **129**, 3570.
8. J. S. Pedersen, *Adv. Colloid Interface Sci.*, 1997, **70**, 171.
9. P. R. Bevington, *Data Reduction and Error Analysis for Physical Sciences*, McGraw-Hill, New York, **1969**.