Electronic Supporting Information

A charge-adaptive nanosystem for prolonged and enhanced *in vivo* antibiotics delivery

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1. Materials

Monomethoxy poly(ethylene glycol) (CH_3O−PEG−OH; M_w = 2000; M_w/M_n = 1.05; 99%, Fluka). ε-Caprolactone (ε-CL, 99%, J&K) was dried over calcium hydride and then purified by distillation under reduced pressure before use. Toluene was dried over calcium hydride and purified by distillation in the presence of Na. $CHCl_3$ was dried over calcium hydride and purified by distillation. $Sn(Oct)_2$ (95%), acryloyl chloride (95%), Hexane-1,6-dioldiacrylate (HDD, 99%), 4,4′-trimethylene dipiperidine (TDP, 97%) and Vancomycin hydrochloride were purchased from J&K and used as received. 6-Carboxytetramethylrhodamine (TAMRA) was purchases from Aladdin. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC·HCl; ≥98%, Fluka), N-hydroxysuccinimide (NHS; ≥97%, Fluka).

All bacteria culture reagents and cells culture reagents were provided by Gibco (GrandIsland, USA). Four- to five-week-old healthy female BALB/c nude mice were obtained from Vital River Laboratories (Beijing, China) and fed in a germ-free environment. All the animal experiments were performed according to the protocol approved by IRM-CAMS.

2. Synthesis of Vanco-TAMRA

TAMRA (2.0 mg, 4.3 μmol) was dissolved in the mixture of 2 ml of DMF and 4 mL of DMSO, followed by addition of the solution of EDC·HCl and NHS in DMSO. After incubation at room temperature for 2 h, the solution of Vancomycin (13 mg, 8.7 μmol) with 2 μL of TEA in DMSO was added. After stirring overnight, the mixture was transferred to a dialysis bag (MWCO 1000) and dialyzed against distilled water for 3 days. Vanco-TAMRA was obtained by lyophilisation.

Vanco-TAMRA was characterized by Electron spray ionization (ESI) mass spectrometry (VG-ZAB-HS spectrometer) for investigating the mass value. As shown in Fig. S4, m/z calculated for [M] and [M+NHS-H_2O] were 1860.65 and 1957.65 respectively.

3. Synthesis of PEG-*b*-PCL

PEG-*b*-PCL was synthesized through ring-opening polymerization of ε-CL using PEG-OH as the initiator according to the previous report. Briefly, PEG_{113}-OH (1.0 g, 0.2 mmol) and ε-CL(0.46 g, 4 mmol) was dissolved in 10 mL of toluene, and then one drop of $Sn(Oct)_2$ was added into the solution. After freeze-degas-thaw cycles for three times, the reaction mixture was stirred at 110 °C for 12 h. Then the solvent was

removed and the crude product was dissolved in an appropriate amount of dichloromethane, followed by precipitation into excess diethyl ether. The precipitate was dried under vacuum.

PEG-*b*-PCL was characterized by ^{1}H NMR (Fig. S5 and S6), the degree of polymerization (DP) of CL was estimated to be 18 by calculating the peak integration ratio of -OC*H*$_2$C*H*$_2$- protons of PEG at 3.59 ppm and -OCOC*H*$_2$- protons of PCL at 2.30 ppm. PDI is about 1.14 (GPC using THF as eluent) as shown in Fig. S8.

4. Synthesis of PEG-*b*-PCL-*b*-PAE

PEG-*b*-PCL-*b*-PAE was synthesized by a Michael-type addition polymerization of PEG-*b*-PCL monoacrylate, HDD and TDP. PEG-*b*-PCL-OH (1.0 g, 0.14 mmol) and TEA (0.28 mmol) were dissolved in 20 mLof CH_2Cl_2, and a solution of acryloyl chloride in CH_2Cl_2 was added dropwise under argon atmosphere at 0 °C. The reaction mixture was stirred overnight at room temperature. The mixture was washed with saturated sodium carbonate, and then the organic layer was dried over anhydrous $MgSO_4$. The filtrate was precipitated into excess diethyl ether to obtain PEG-PCL monoacrylate. PEG-*b*-PCL monoacrylate (0.5 g, 0.07 mmol), HDD (0.21 g, 0.91 mmol) and TDP (0.20 g, 0.97 mmol) were dissolved in 7 mL of $CHCl_3$. After stirred for 72 h at 55 °C, the solution was precipitated into excess diethyl ether to obtain PEG-*b*-PCL-*b*-PAE.

PEG-*b*-PCL-*b*-PAE was characterized by ^{1}H NMR as shown in Fig. S7. The DP of polymerization of PAE was determined to be 12 by calculating the peak integration ratio of -OC*H*$_2$C*H*$_2$- protons of PEG at 3.59 ppm and -OC*H*$_2$CH$_2$- protons of PCL at

4.15 ppm. PDI is about 1.33 (GPC using THF as eluent) as shown in Fig. S8.

5. Characterization

^{1}H NMR spectra were recorded on a Varian UNITY-plus 400 M NMR spectrometer at room temperature with tetramethylsilane (TMS) as an internal standard. Electron spray ionization (ESI) mass spectrometry was performed on a VG-ZAB-HS spectrometer (VG Company, England using the Fast Atom Bombardment (FAB) method. The number-average molecular weight (M_n) and weight-average molecular weight (M_w) were measured by gel permeation chromatography (GPC) at 25 °C with a Waters 1525 chromatograph equipped with a Waters 2414 refractive index detector. GPC measurements were carried out using THF as eluents with a flow rate of 1.0 mL/min, respectively. Polystyrene and PEG standards were used for calibration. Dynamic light scattering (DLS) experiments at a 90° scatter angle were performed on a laser light scattering spectrometer (BI-200SM) equipped with a digital correlator (BI-9000AT) at 636 nm at required temperature. All samples were obtained by filtering through a 0.45 μm Millipore filter into a clean scintillation vial. The zeta potential values were measured on a Brookheaven ZetaPALS (Brookheaven Instrument, USA), using phosphate buffer (PB) solution (0.01 M) with a pH range from 5.0 to 7.4 as the background buffer. The instrument utilizes phase analysis light scattering at 37 °C to provide an average over multiple particles.

6. Preparation of micelles

2 mg of PEG-*b*-PCL-*b*-PAE was dissolved in 1 mL of DMSO/ethyl acetate solution (15/85, v/v), followed by addition of 0.1 mL of Vanco-TAMRA (0.40 mg)

solution in water. Then the mixture was sonicated for 5 min to form emulsion. The whole solution was then emulsified into 4 ml of NaCl solution in water (10 %, w/v) under sonication for 5 min. This concentrated double emulsion was added into 16 mL of NaCl solution in water (5 %, w/v). After evaporation at room temperature for 12 h, the nanoparticle solution was transferred to dialysis bag (MWCO 3500) and dialyzed against deionized water for 48 h. The micelle solution was concentrated ultrafiltration before use.

Vanco-TAMRA encapsulated PEG-*b*-PCL micelle was prepared with similar procedure. The parameters of drug encapsulation of PEG-*b*-PCL/Van and PEG-*b*-PCL-*b*-PAE/Van were shown in Table S1. Drug loading content (DLC) and drug loading efficiency (DLE) of PEG-*b*-PCL/Van and PEG-*b*-PCL-*b*-PAE/Van nanoparticles was measured by using a fluorescence spectrometer (excitation at 550 nm and emission at 580 nm). Briefly, the micelle solution was diluted into 50-fold volume of DMF and the Vanco-TAMRA concentration was measured by calculating the height of emission peak at 580 nm according to the calibration curve of Vanco-TAMRA

7. Transmission electron microscope (TEM)

The morphology of drug-loaded micelles were examined using transmission electron microscope (TEM). Micelles were prepared as above descripted. 10 μL of the samples were dropped on a copper grid and incubated for 60 s and then rinsed with deionized water twice. The sample plates were kept overnight and then were characterized by TEM (JEOL, Japan) at 200 kV.

8. *In vitro* stability study of nanosystem

The *in vitro* stability of the PEG-*b*-PCL-*b*-PAE/Van nanoparticles was performed in PBS buffer (0.01M, pH 7.4) at a Van dose of 100□μg/mL. At designated time points (0 h, 4 h, 12 h and 24 h), 1 mL of sample was withdrawn and the diameters of the nanoparticles were detected by Dynamic light scattering (DLS) spectrometer (BI-200SM).

9. *In vitro* bacteria targeting study

Staphylococcus Aureus (SA) were streaked on a solid LB-agar plate (1.5 % agar) overnight. A single colony from the plate was inoculated into 10 mL of LB medium and was cultured overnight with stable shaking at 200 rpm at 37℃. With appropriate dilution of bacteria product by LB medium, we finally acquired bacteria solution with OD_{600} of 1.0. 1 mL of diluted bacteria solution was centrifugated at 3000 rpm for 5 min to obtain bacteria sediments. After being washed in PBS buffer (pH 7.4), sediments were resuspended in PBS buffer containing different Van formulations (PEG-*b*-PCL-*b*-PAE/Van、PEG-*b*-PCL/Van and free-Van) at different pH values (pH 6.5 and pH 7.4, previously adjusted by 10% Na_2CO_3 and diluted HCl solution) and then combined with constant shaking at 160 rpm for 30 min. After incubation was complete, bacteria were washed by PBS buffer for 3 times to remove the unbound Van formulations. Then bacteria were immobilized in 4% paraformaldehyde for 1 h. After that, 500 μL of DAPI solution (10 μg/mL) was added to stain the DNA of bacteria for 0.5 h. Extra dyes were washed away by centrifuging and sediments were resuspended in 100 μL of PBS. 10 μL of solution was dropped onto slide substrates

then was covered by coverglass. Samples were finally sealed with transparent nail polish. The fluorescence intensities were detected by confocal laser scanning microscopy (CLSM, TCS SP5, Leica, Germany).

***10. In vitro* antibacterial study**

Bacteria were culture in LB medium and were adjusted to appropriate concentration with OD_{600} of 1.0. Van formulations and LB medium were previously adjusted to pH 6.5 and 7.4. The highest concentration of Van formulations was 100 μg/mL. With double dilution in LB medium, they were diluted to 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78, 0.39 and 0.20 μg/mL, respectively, and then being transferred to 96-wells plate. Meanwhile, blank controls without materials were set. 3 μL bacteria liquid (OD600=1) were finally added to each well. After fostering for 24 h at 200 rpm at 37℃, OD_{600} of bacteria solution were measured by microplate spectrophotometer (Varioskan Flash, Thermo, USA).

11. Cell culture and cell viability study

NIH 3T3 cells and 293T cells were cultured in DMEM medium containing 100 U/mL penicillin, 100 μg/mL streptomycin and 10 % fetal bovine serum. The cells were maintained at 37℃ in a humidified incubator with 95 % air and 5 % CO_2. The cytotoxicities of different micelles (PEG-*b*-PCL and PEG-*b*-PCL-*b*-PAE) were determined against NIH 3T3 cells and 293T cells at pH 7.4 by MTT assay. In the MTT assay, NIH 3T3 or 293T cells were seed into 96-well plates at a density of 5000 cells per well in 100 μL DMEM (containing 10 % FBS, 100 U/mL penicillin and 100 μg/mL streptomycin). After cultured for 24 h, both micelles were added to each well

with different concentrations (0, 2, 10, 50, 100, 200, 400 and 500 μg/mL). After 24 h, 25 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) (5 mg/mL) was added to each well and the mixture was incubated for another 4 h, then 150 μL of DMSO was added to dissolve the obtained blue formazan crystals. The absorbance was measured at a wavelength of 570 nm and the viability was expressed as the percentage of the control.

12. Establishment of subcutaneous inflammation model

A colony from *SA*-cultured agar plate was picked into 10 mL of LB medium and then was cultured for 12 h vibrating at 200 rpm at 37℃. When its OD600 reached 1.0, 1 mL bacteria solution were sucked into 1.5 mL eppendorf tube, then centrifugated at 3000 rpm for 5 min. Supernatant was discarded and 1 mL of PBS buffer was added. After a second time of centrifugation, the bacterial sediments were resuspended in 100 μL of PBS. Every BALB/c nude mice received a subcutaneous injection of 50 μL solution on the backside.

13. *In vivo* imaging of TAMRA-Van on skin inflammation model

Model inflammation mice were divided into three groups (free-Van, PEG-*b*-PCL/Van and PEG-*b*-PCL-*b*-PAE/Van) 24 h after subcutaneous injection when mices skin inflammation came into being. The mice were intravenously injected with 200 μL of corresponding Van formulations at a Van dose of 100 μg/mL. At different time points (2 h, 12 h and 24 h), the intensities of different mice were detected by the Maestro EX fluorescence imaging system (CRi, Inc.).

14. Statistical analysis

All the statistical analyses were performed using SPSS 19.0 software. The statistical significance of differences was determined by *LSD-t* test. $p < 0.05$ and $p < 0.01$ were used to show statistical difference and significant difference, respectively.

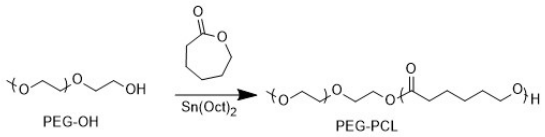

Fig. S1 Synthesis of PEG-*b*-PCL

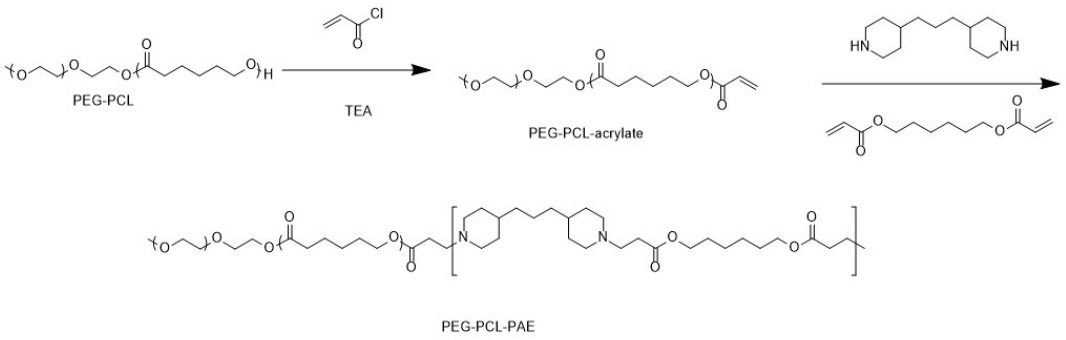

Fig. S2 Synthesis of PEG-*b*-PCL-*b*-PAE

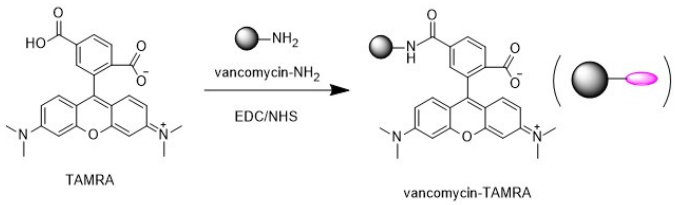

Fig. S3 Synthesis of Vanco-TAMARA

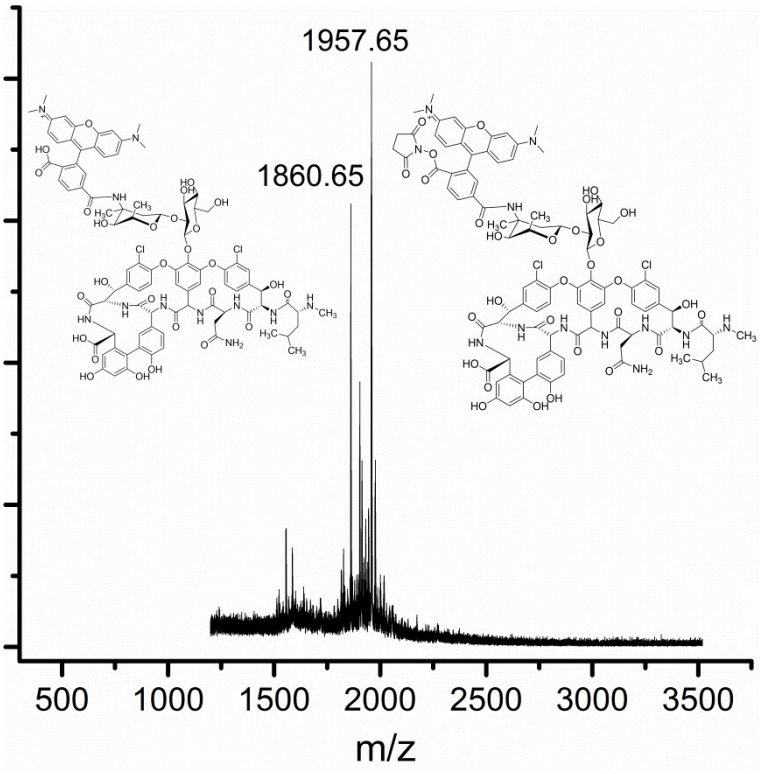

Fig. S4 Mass spectrum of Van-TAMRA

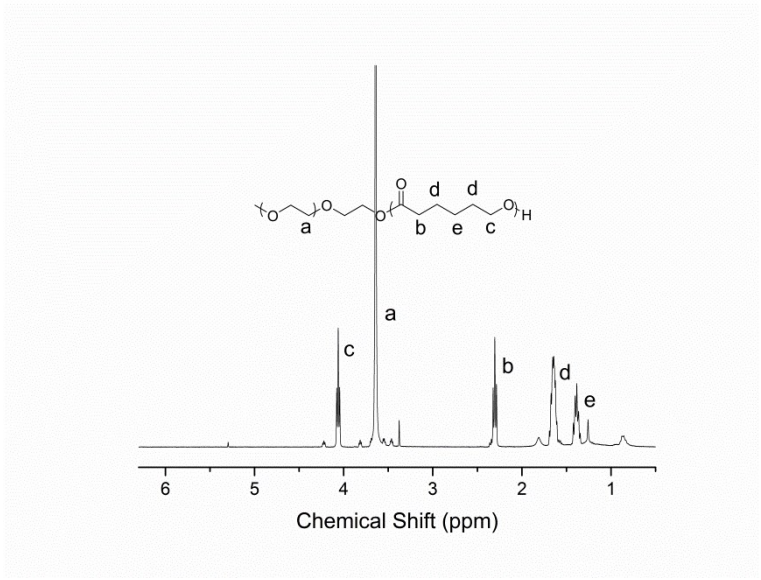

Fig. S5 ^{1}H NMR of PEG_{113}-*b*-PCL_{18}

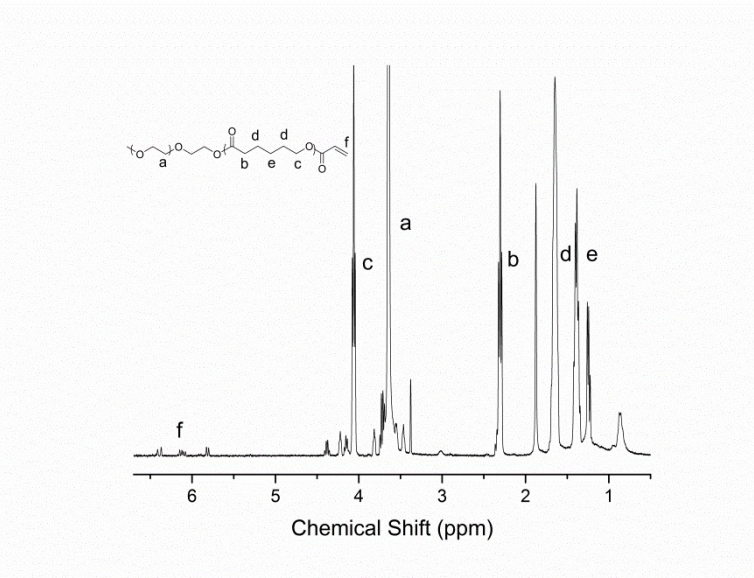

Fig. S6 ^{1}H NMR of PEG_{113}-*b*-PCL_{18}-acrylate

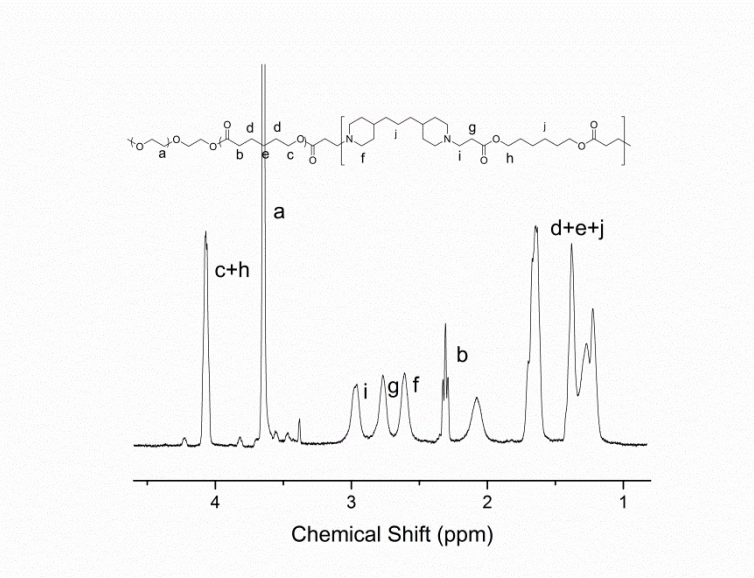

Fig. S7 ^{1}H NMR of PEG-*b*-PCL-*b*-PAE

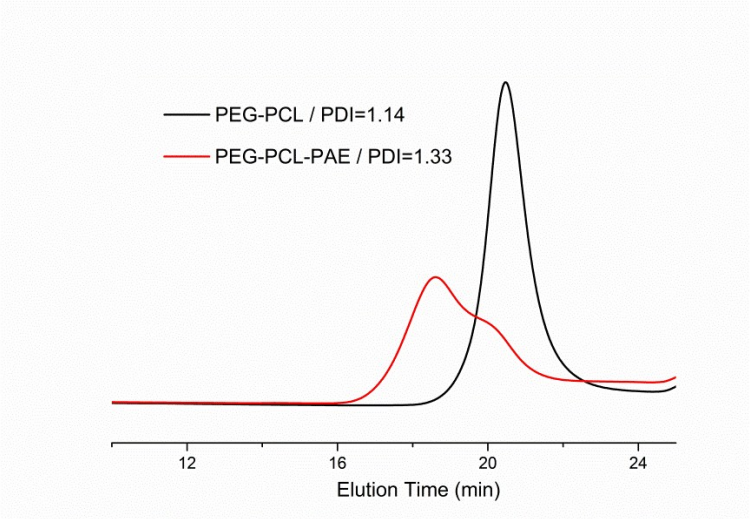

Fig. S8 GPC spectrum of PEG-*b*-PCL and PEG-*b*-PCL-*b*-PAE

Table S1 Parameters of drug encapsulation of PEG-*b*-PCL/Van and PEG-*b*-PCL-*b*-PAE/Van nanoparticles

sample	DLC (%)[a]	DLE (%)[b]	nanoparticle yield (%)	size (nm)[c]	PDI[c]
PEG-*b*-PCL/Van	7.8	40.6	90.1	61±2.5	0.12
PEG-*b*-PCL-*b*-PAE/Van	9.2	45.4	90.8	53±3.0	0.15

[a] Determined by using a fluorescence spectrometer (excitation at 550 nm and emission at 580 nm).

[b] Feed ratio of Vanco-TAMRA to polymers was 20 mg/100 mg.

[c] Detected by dynamic light scattering (DLS) laser spectrometer (BI-200SM) at 25 °C.

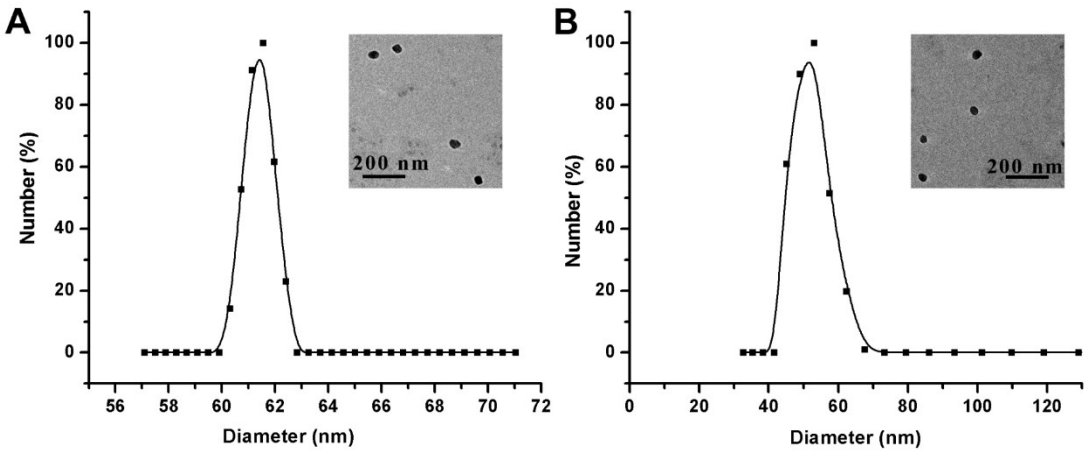

Fig. S9 Diameter (DLS) and TEM Images of PEG-*b*-PCL/Van (A) and PEG-*b*-PCL-*b*-PAE/Van (B)