Electronic Supplementary Information (ESI) for

Homopolymer vesicles with a gradient bilayer membrane as drug carriers

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Experimental Section

Matierials. 2-(2-Ethoxyethoxy)ethyl acrylate (EEA) was purchased from Aladdin and passed through a column filled with silica gel and alumina B to remove the inhibitor before use. Doxorubicin (DOX) was purchased from Xingcheng Chempharm Co., Ltd., China and used as its hydrochloride form. Other reagents and solvents were purchased from Sinopharm Chemical Reagent Co., Ltd and uesd without further purification.

Characterization. Gel permeation chromatography (GPC) analysis was carried out with a Waters Breeze 1525 GPC analysis system with two PL mix-D column, using DMF with 0.5 M LiBr as eluent at a flow rate of 1.0 mL/min and 80 ^{o}C. PEO calibration kit (purchased from TOSOH) was used as the calibration standard.

Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded using a Bruker AV 400 MHz spectrometer, with $CDCl_3$ or D_2O as solvent and TMS as standard. When D_2O/H_2O solvent mixture was used, the "water suppression" software option was selected.

Transmission Electron Microscopy (TEM) images of PEEA vesicles were obtained using a JEM-2100F electron microscope operating at an acceleration voltage of 200 kV. 4.0 μL of homopolymer vesicle solution at 10 ^{o}C was dropped onto a carbon-coated copper grid. The water droplet was blotted after 10 seconds. This process was repeated for at least 3 times. The copper grid was then stained with 1% aqueous phosphotungstic acid solution at pH 7.0.

UV-vis study. The optical transmittance spectra of aqueous vesicle solutions were recorded using a UV–vis spectrophotometer (Shanghai Precision & Scientific Instrument Co., Ltd., UV759S).

Fluorescence spectroscopy. Fluorescence intensities of aqueous samples were measured with a fluorescence spectrometer (Thermo Scientific; Lumina).

Dynamic light scattering (DLS) was used to determine the hydrodynamic diameter (D_h) and polydispersity of vesicles formed from homopolymers in aqueous solution. The field correlation function, $g^{(1)}(t)$, is calculated from the measured intensity autocorrelation function, $g^{(2)}(t)$ through the Siegert equation (Eq. 1).

$$g^{(2)}(\tau\text{-}1)=\beta|g^{(1)}(\tau)| \quad (1)$$

In a polydispersed spheres solution , the $g^{(1)}(t)$ (first-order electric field correlation function) is related to G(Γ) (the line width distribution function) by

$$g^{(1)}(t)=\int_0^\infty G(\Gamma)e^{-\Gamma t}d\Gamma \quad (2)$$

where t refers to the delay time. Both the cumulant analysis and the CONTIN analysis are employed as data processing methods. The cumulant analysis (Eq. 3) can be applied to describe logarithm of $g^{(1)}(t)$ as a series expansion of time (t). <Γ>, the first cumulant, is the decay rate of the process which yields the z-averaged diffusion coefficient. The second cumulant (μ_2) is correlated to the second moment of the distribution of relaxation times by Eq. 4.

$$\ln[g^{(1)}(t)]=-<\Gamma>t+(\frac{\mu_2}{2})t^2-(\frac{\mu_3}{6})t^3+\ldots \quad (3)$$

$$\mu_2=\int_0^\infty G(\Gamma)(\Gamma\text{-}<\Gamma>)^2 d\Gamma \quad (4)$$

where G(Γ) is a line width distribution function and Γ is given by

$$\Gamma=Dq^2 \quad (5)$$

where D is the translation diffusion coefficient, and q [= $(4\pi n/\lambda_0)\sin(\theta/2)$] is the scattering vector, θ is the scattering angle, n is the refractive index of the solvent, and λ_0 is the wavelength of the incident light.

<Γ> and μ_2 can be acquired directly by the cumulant methods and meanwhile the hydrodynamic radius distribution $f(R_h)$ can be obtained by the CONTIN program in the correlator. Then with the assist of the Laplace inversion, the line width distribution function

G(Γ) can be figured out based on the measured $g^{(1)}(t)$. For a diffusive relaxation, Γ can be interrelated to D (the translational diffusion coefficient) by Eq. 5. Based on the mathematical relation mentioned above, G(Γ) can be converted to $f(R_h)$ according to Eq. 6, namely the Stokes-Einstein equation.

$$R_h = \frac{k_B T}{6\pi\eta D} \quad (6)$$

where k_B is the Boltzmann constant, T is the absolute temperature, and η is the viscosity of the solvent.

The hydrodynamic diameters of different concentrations of aqueous homopolymer vesicle solutions at different temperatures were characterized by ZETASIZER Nano series instrument (Malvern Instruments ZS 90). The scattering angle was fixed at 90°. Data processing was carried out using cumulant analysis of the experimental correlation function and analyzed using Stokes-Einstein equation to calculate the hydrodynamic diameters of homopolymer vesicles. All the aqueous aggregates solutions were analyzed using disposable cuvettes.

Zeta potential. The electrophoretic mobilities (U_E) of aqueous samples are calculated by definition:

$$U_E = \frac{v}{E}$$

Here, E stands for electric field strength and v stands for the the speed of the particles. v is determined by the parameters such as properties of charged particles, voltage gradient, solvent properties and temperature, etc. In this case, the vesicle solutions are largely dilute and the direct effect of temperature is limited, so the U_E is mianly determined by properties of charged particles.

The zeta potential of the particle can be obtained by the application of the Henry equation:

$$U_{\mathrm{E}} = \frac{2\varepsilon z f(K_{\alpha})}{3\eta}$$

Where z, ε, η, and $f(\mathrm{K}_{\alpha})$ stand for zeta potential, dielectric constant, viscosity, and Henry's function, respectively. In this case, electrophoretic determinations of zeta potential are made in aqueous media and moderate electrolyte conceration and $f(\mathrm{K}_{\alpha})$ is 1.5. The calculation of zeta potential from mobility is straightforward for system that fits the Somluchowski model.

Synthesis of Chain Transfer Agent (CTA) of DDMAT.

2-(Dodecylthiocarbonothioylthio)-2-methylpropanoic acid (DDMAT) was synthesized according to our previously reported method.[1] As shown in Scheme S1, a suspension of K_3PO_4 (5.14 g, 24.2 mmol) in acetone (125 mL) was added with 1-dodecane thiol (5.00 g, 24.2 mmol) and kept stirring for 30 min. CS_2 was then injected into the mixture and the solution became yellow immediately. After stirring for another 30 min, 2-bromo-2-methylpropionic acid (3.71 g, 21.8 mmol) was added and the reaction mixture was kept stirring for 20 h at room temperature. The solvent was removed by rotary evaporation at 35 °C and the orange residue was extracted into CH_2Cl_2 (3× 200 mL) from 1.0 M HCl solution (200 mL). Afterwards, the organic phase was washed with water (2× 200 mL) and brine (200 mL) successively and dried over anhydrous $MgSO_4$ overnight. The solvent was then evaporated under reduced pressure at 35 °C and the residue was then recrystallized from *n*-hexane twice to yield a bright yellow crystal.

Synthesis of PEEA homopolymer via RAFT. In a general procedure (Scheme S1), a single neck flask with a magnetic flea was added with EEA (2.000 g, 10.6 mmol), DDMAT (77.5 mg, 21.3 × 10^{-2} mmol), and dioxane (4.0 mL). Then the flask was subjected to argon bubbling to deoxygenate with stirring for 30 min. AIBN (3.89 mg, 4.25×10^{-2} mmol) was rapidly added in the flask with additional argon bubbling for 5 minutes. The sealed flash with argon protected is subsequently immersed into an oil bath at 80 ^{o}C. The polymerization was

teminated after 48 hours by cooling down the system to room temperature and opening the flask to air. The dioxane was removed under vacuum. The crude polymer was then dissolved in dichloromethane and precipitated in n-hexane. This dissolution/precipitation procedure was repeated twice. Then the polymer was dried under vacuum at 20 ^{o}C. ^{1}H NMR spectrum (Fig. S2A): Peaks c (3.4 ppm), f (1.9 ppm), g (1.3 ppm) and i (0.8 ppm) are assigned to the protons of DDMAT end group. Peaks d+e at 2.4~1.8 ppm are from PEEA back bone. Peaks a, b, h at 4.2 ppm, 3.9~3.5 ppm and 1.3 ppm are attributed to the protons of OEs. The homopolymer has hydrophobic fractions as low as 18 wt% (3.8 wt% originates from DDMAT end group functionality and 14.2 wt% from hydrophobic backbone).

Preparation of homopolymer vesicles. The following protocol was adopted. PEEA homopolymer (1.5 mg, varies with the targeted concentration) was first dissolved in THF (3.0 mL). Deionized water (6.0 mL) was dropwise added within 10 min with vigrous stirring. Then additional 6.0 mL of deionized water was added at one stroke. The solution was allowed to dialysed against 500 mL of deionized water for 2 days (renew fresh water for 6 times) to remove THF.

Critical vesiculation concentration (CVC). The CVC of $PEEA_{37}$ homopolymer was determined by a fluorescence spectrometer using pyrene as a hydrophobic probe.[2] 4.0 mg of pyrene was dissolved in 10 mL of acetone and 5 μL pyrene solution was added to each cuvette. The acetone was allowed to evaporate at room temperature. $PEEA_{37}$ homopolymer vesicle solution was diluted to various concentrations ranging from 0.488 to 62.5 μg/mL by deionized water. Then 4.0 mL of diluted aqueous homopolymer solution was added into each pyrene-containing cuvette. The solutions were kept at room temperature and equilibrated by stirring overnight. The CVC was estimated by extrapolating the fluorescence intensity of pyrene (λ_{ex} = 334 nm and λ_{em}= 372 nm) at various polymer concentrations.

Cloud Point Measurements. The cloud point of the aqueous homopolymer solution was measured using a UV-vis spectrophotometer. The transmittance of homopolymer solution in deionized water at 510 nm was monitored as a function of temperature (one heating/cooling cycle at a rate of 0.3 ^{o}C/min). It is necessary to clean the ektexine of the cuvette by degreasing cotton because rapid generation of mists by the temperature variation betwween the cuvette and air when the solution is at low temperatures.

Cytotoxicity Tests. Cellular viabilities of both normal cells and cancer cells were determined using the Cell Counting Kit-8 assay (CCK-8, Dojindo, Japan). Liver normal cells (L02 cells) were seeded with equal density in each well of 96-well plates (4000 cells/well) in 100 μL of Dulbecco's Modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) for 24 h at 37 °C in a humidified 5% CO_2-containing atmosphere. Then 20 μL of PEEA vesicle solution with final concentrations of 62.5 μg/mL, 125 μg/mL, 250 μg/mL, 500 μg/mL, and 1000 μg/mL, respectively, was added and incubated with cells for additional 48 h. Untreated cells served as a control group. At the end of the treatment, CCK-8 dye was added to each well and the plates were incubated for additional 1 h at 37 ^{o}C. Subsequently, the absorbance was measured by dual wave length spectrophotometry at 450 nm and 630 nm using a microplate reader. Each treatment was repeated five times. The relative cell viability (%) was determined by comparing the absorbance at 450 nm with control wells containing only cell culture medium.

Cellular viability test of PEEA vesicles by using cancer cells (HeLa cells) was also performed at different treatment times (Fig. S11).

Drug loading and release of PEEA vesicles. The loading and controlled release of drug was achieved accroding to the flollowing protocol. 20.0 mg of $PEEA_{37}$ homopolymer was

dissolved in 15.0 mL of THF in a flask. Then 5.0 mg of DOX was added after 10 mins. After 5 mins, 20 mL of water was dropped into the flask within 10 mins. Then additional 20 mL of water was added at one stroke and stirred overnight. THF was removed under vacuum before removing free drug by dialysis against 500 mL of water for 2 h (renew fresh water every 30 min). The above process were conducted at 25 ^{o}C and the final volume of aqueous vesicle solution is 47.0 mL. The DOX-loaded mixture was diluted by 20 times to calculate the weight of drug encapsulated in vesicles via a fluorescence spectrometer (λ_{ex} = 461 nm and λ_{em} = 591 nm) and the cumulative release curve of DOX was obtained. The calibration curve of the fluorescent intensity of DOX at various concentrations in 0.01 M tris buffer at pH 7.4 and 25 ^{o}C are shown in Fig. S10. The final drug release experiment was carried out by dialyzing 10.0 mL of DOX-loaded vesicles (no dilution) in the dialysis tube against 150 mL of tris buffer (0.01 M; pH 7.4) in a beaker (250 mL), at 37 °C and 190 r/min of stirring rate. The volume of liquid in the beaker (outside of the dialysis tube) was ensured around 150 mL during the measurement. At different time intervals, the liquid in the beaker were measured by a fluorescence spectroscopy. The drug loading content (DLC) and drug loading efficiency (DLE) were calculated according to the following equations:[3]

DLC (%) = (weight of drug encapsulated in vesicles / weight of polymer) × 100%

DLE (%) = (weight of drug encapsulated in vesicles / weight of drug in feed) × 100%

Scheme S1 Synthesis of the chain transfer agent DDMAT and PEEA homopolymer by RAFT.

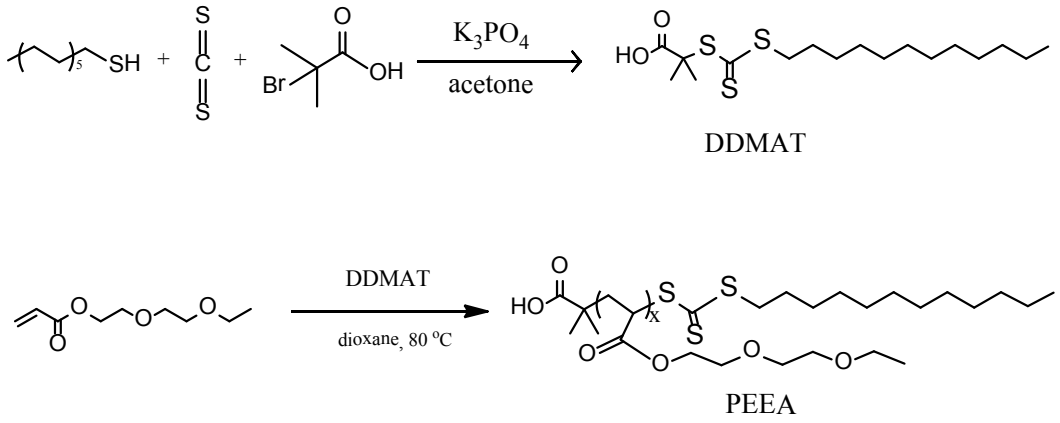


Fig. S1 ^{1}H NMR spectrum of 2-(dodecylthiocarbono-thioylthio)-2-methyl propanoic acid (DDMAT) in $CDCl_3$.

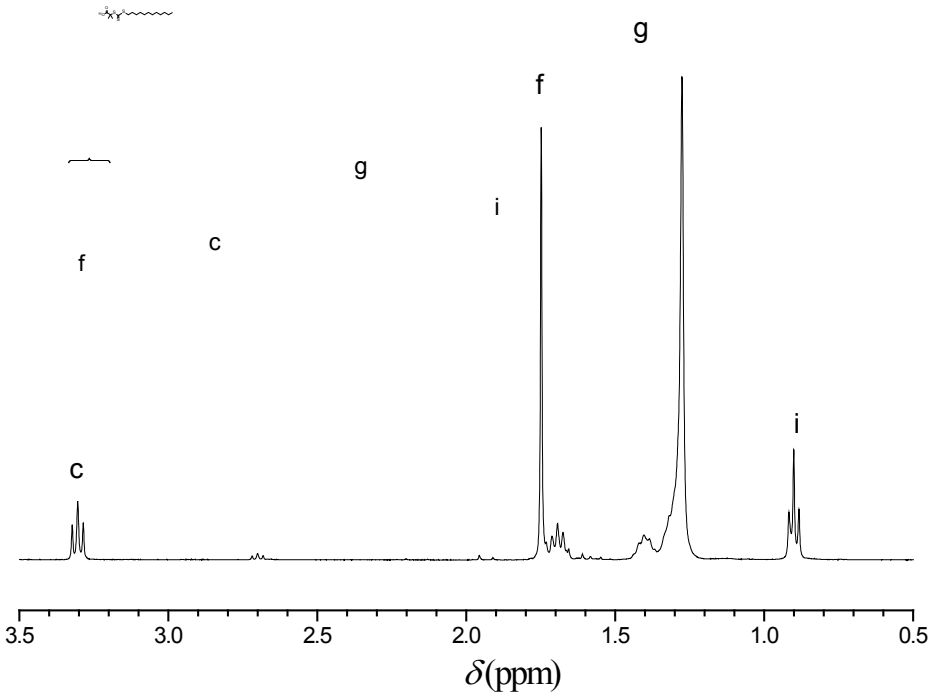


Fig. S2 ^{1}H NMR spectra of (**A**) poly(2-(2-ethoxyethoxy)ethyl acrylate) (PEEA) homopolymer chains in $CDCl_3$ and (**B**) PEEA homopolymer vesicles in H_2O/D_2O at 20 ^{o}C.

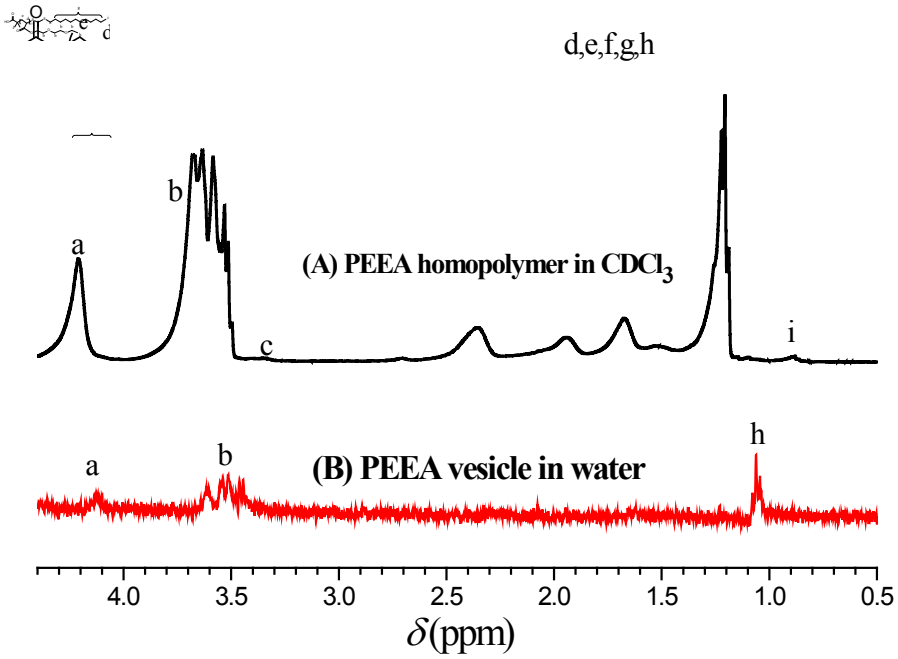
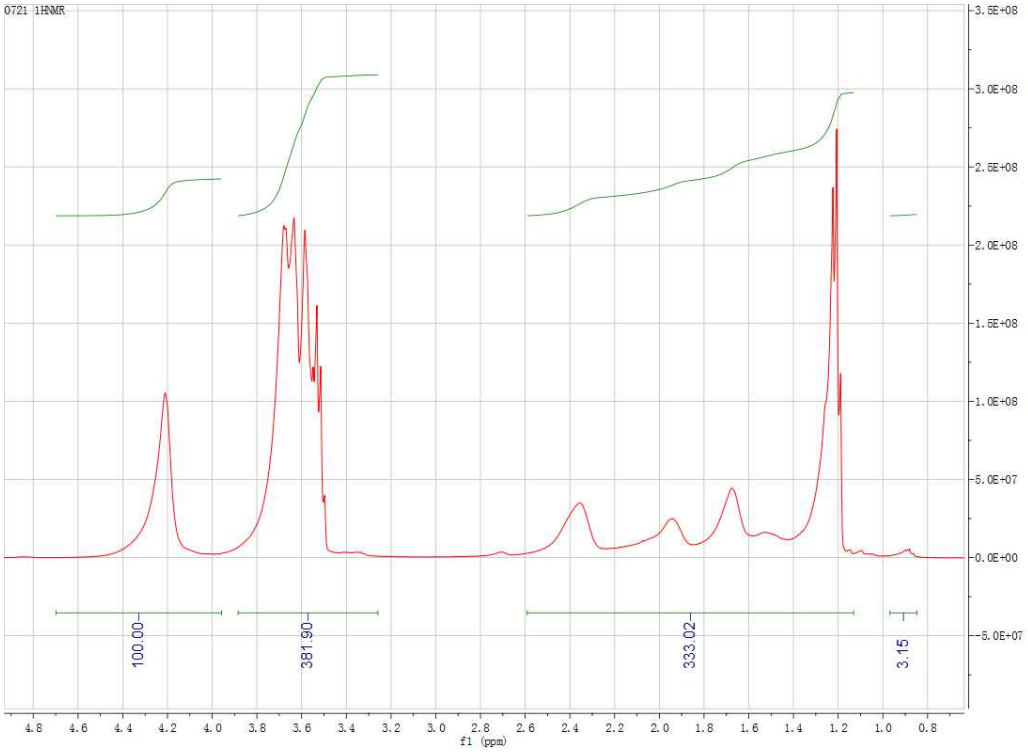


Fig. S3 ^{1}H NMR spectrum with integrals of PEEA in $CDCl_3$.



Calculation of the degree of polymerization of EEA

Table S1. The integral areas of different peaks and the degree of polymerization (*DP*) of EEA

Polymer	A_a	A_{d+e+h}	$A_{d+e+f+g+h}$	A_{f+g}	x
$PEEA_{37}$	100	300	333.0	35.8	37

The integral area of peak a was set to be 100 as the internal reference. The integral areas of other peaks are listed in Table S1. In a typical calculation, DP was determined by comparing A_a with A_{f+g}:

$x = (A_a/A_{f+g}) \times 26/2 = 74.12/2 = 37.06 \approx 37$

Calculation of the contour length of PEEA

The contour length of PEEA was calculated by accumulating the length of carbon-carbon covalent bonds at a specific angle:

The angle of carbon-carbon covalent bonds is 109.28^o.

$l_{PEEA} = l_{c\text{-}c} \times DP \times 2 \times \cos((180^o - 109.28^o)/2) = 0.154\ nm \times 37 \times 2 \times \cos(35.36^o) \approx 9.3\ (nm)$

The vesicle membrane thickness of 18.5 nm from TEM images is comparable to the double contour length ($2 \times 9.3 = 18.6$ nm) of the PEEA block, which suggests that these chains are aggregated to form a bilayer structure within the vesicle membrane.

The length of CTA end group is roughly calculated by the contour length of *n*-dodecyl group:

$l_{CTA} = l_{c\text{-}c} \times 11 \times \cos(35.36^o) = 0.154\ nm \times 11 \times 0.8155 \approx 1.38\ (nm)$

Fig. S4 Gel permeation chromatography (GPC) trace of PEEA homopolymer.

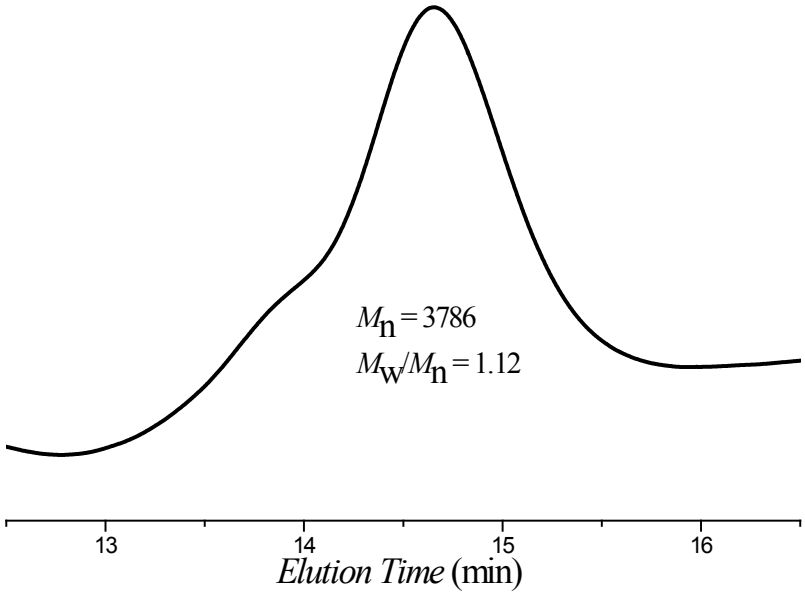


Fig. S5 Fluorescence intensity of pyrene (λ_{ex} = 334 nm and λ_{em}= 372 nm) as a function of the concentration of PEEA in water. This experiment confirmed the CVC of PEEA is 11.9 μg/mL.

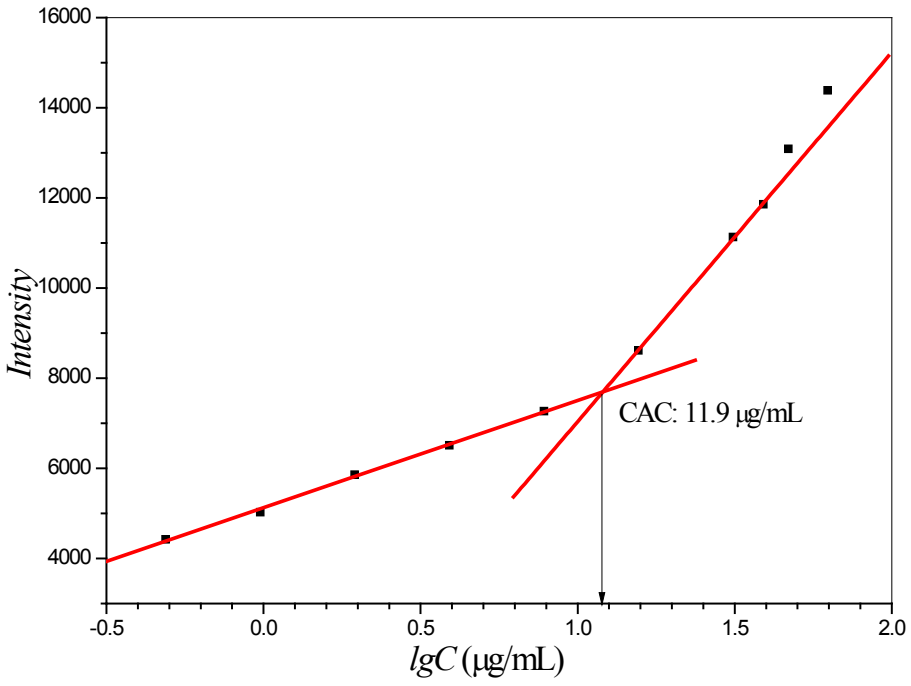
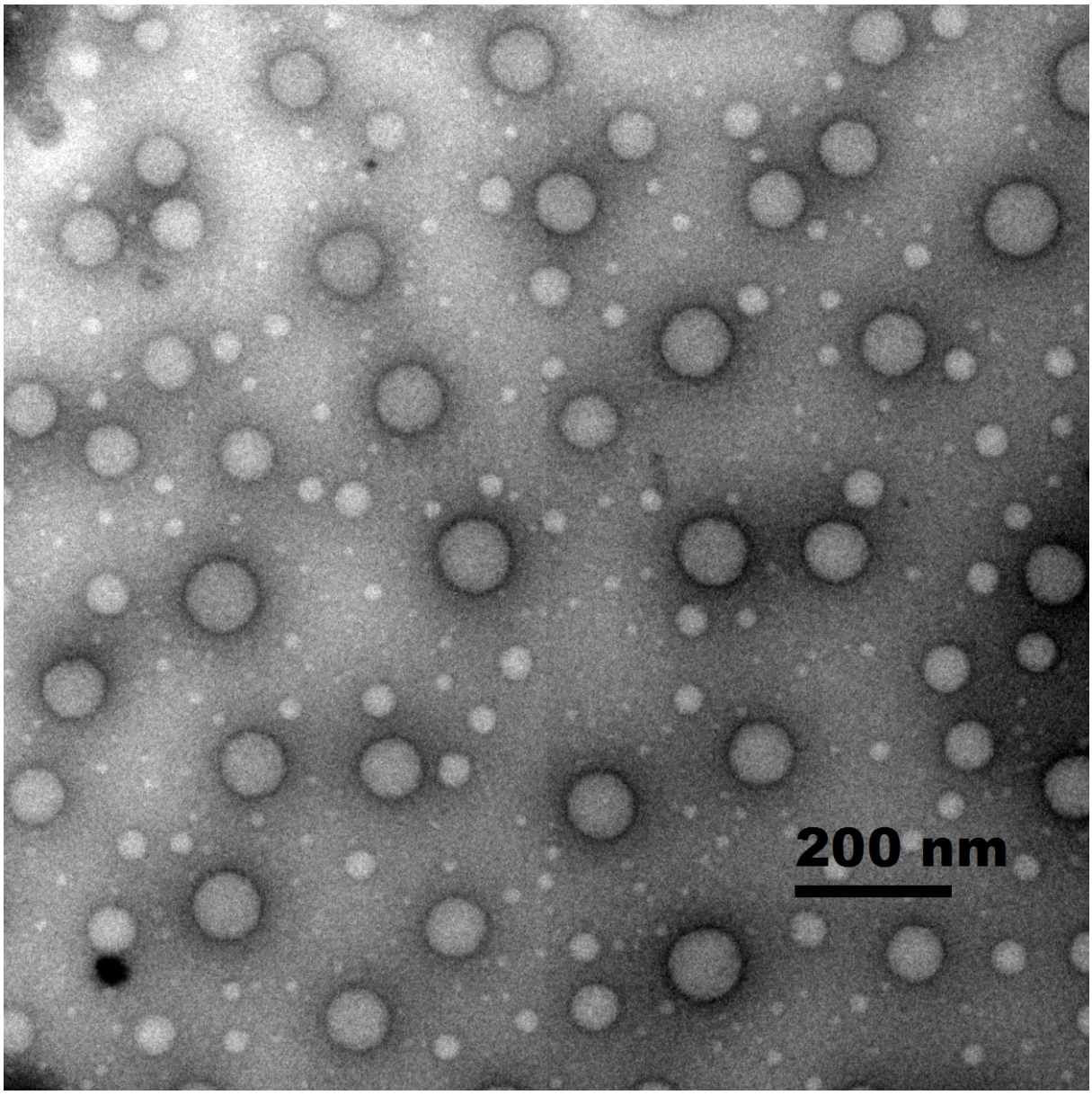
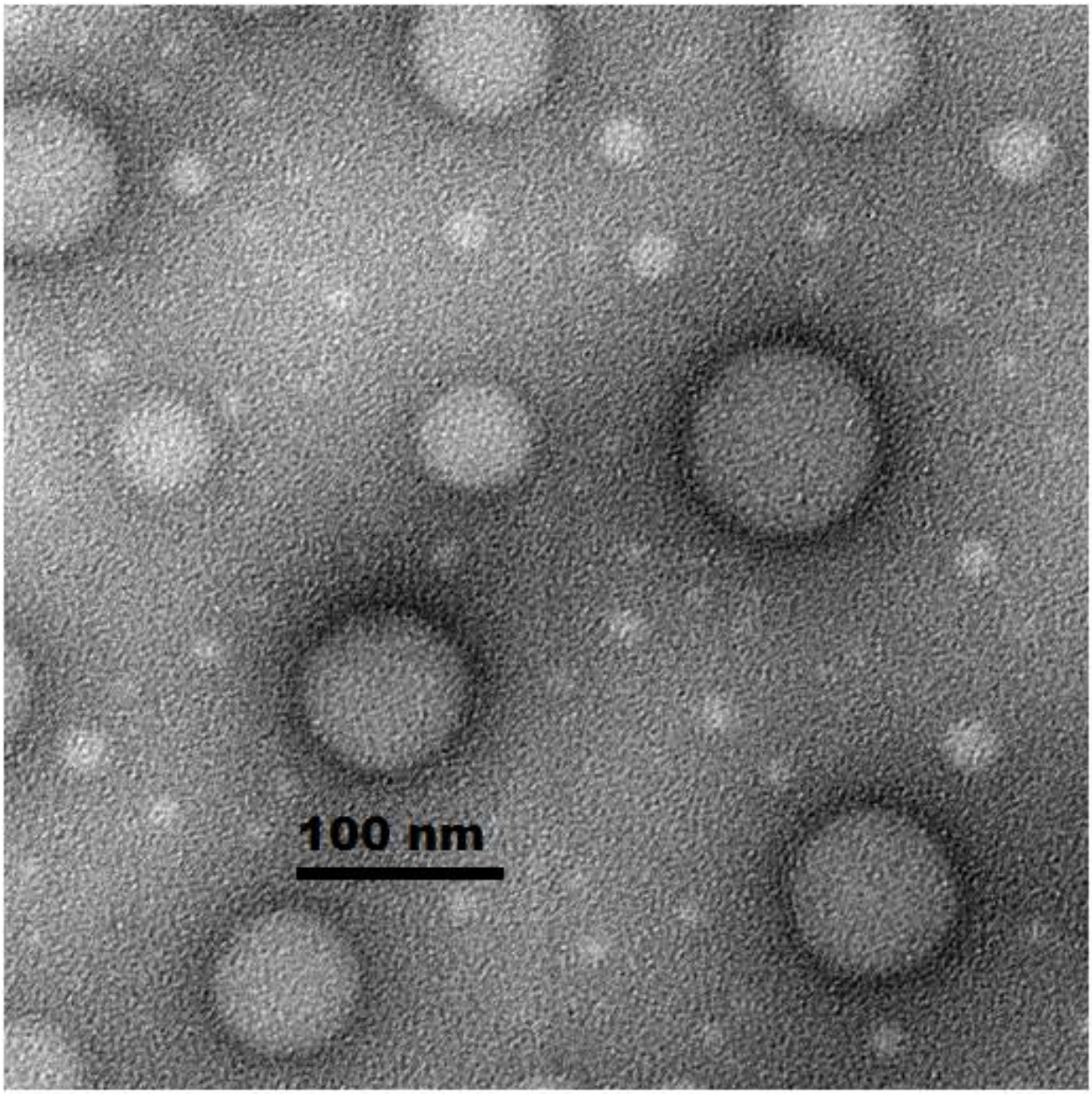


Fig. S6 More TEM images of PEEA homopolymer vesicles at 10 ^{o}C.

A) A magnified image of Fig. 1A in the main text. The hollow vesicular structure has a mean diameter of ca. 42 nm.



B) A magnified image of Fig. 1B in the main text.



C) TEM image of PEEA vesicles at a sligh defocus state. The mean diameter of vesicles calculated from this image is 47.7 nm. These hollow vesicular structures indicated that the appeared white dots in Fig. 1 in the main text and Fig. S6A-B were small vesicles. For example, a small vesicle (aforementioned white a white dot) has a diameter of ca. 40 nm and a membrane thickness of ca. 13 nm by analyzing the intensity of the electron transmittance along the scan line of **1**. However, the bigger vesicle has the same membrane thickness of 18 nm as that in Fig. 1 in the main text. The thinner membrane of the smaller vesicles than that of the bigger vesicles may be related to the polydispersity of PEEA homopolymers. That is to say, the shorter PEEA chains form smaller vesicles with a thinner membrane.

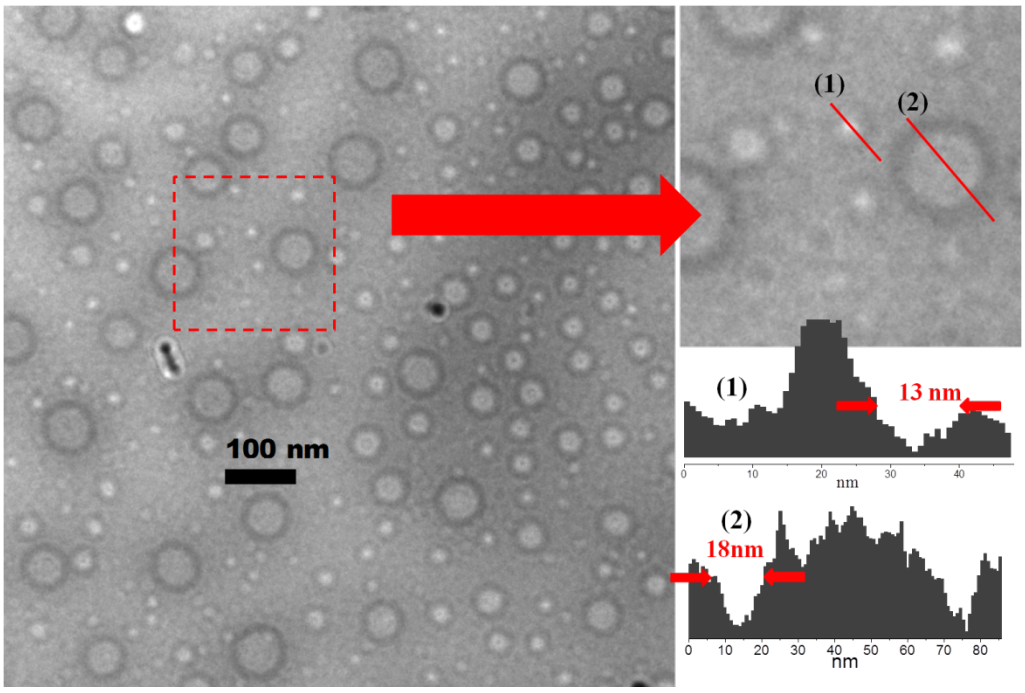


Fig. S7 More AFM images of PEEA homopolymer vesicles at 10 ^{o}C (A-C) and 25 ^{o}C (D-F). The diameter/height ratio is ca. 10, which is much bigger than that of a solid particle, suggesting the PEEA homopolymer vesicles have a hollow structure.

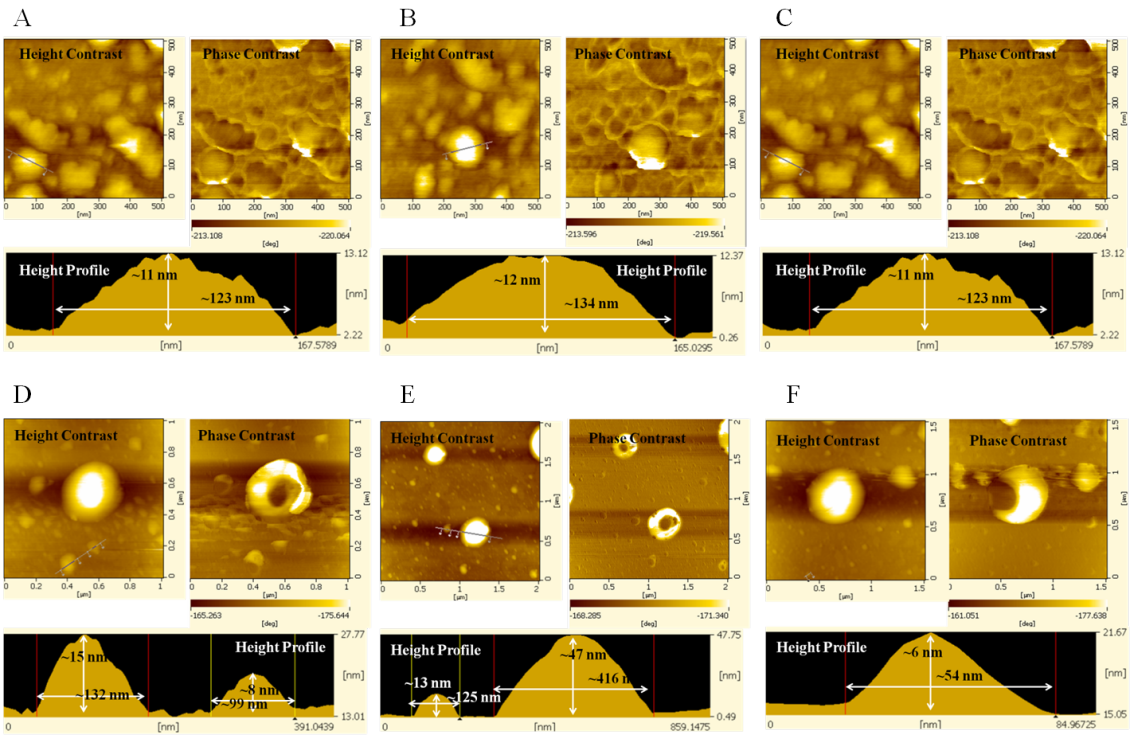


Fig. S8 Plot of transmittance anginst temperature of PEEA vesicles at a C_{ini} of 0.5 mg/mL in a heating/cooling cycle (λ = 510 nm).

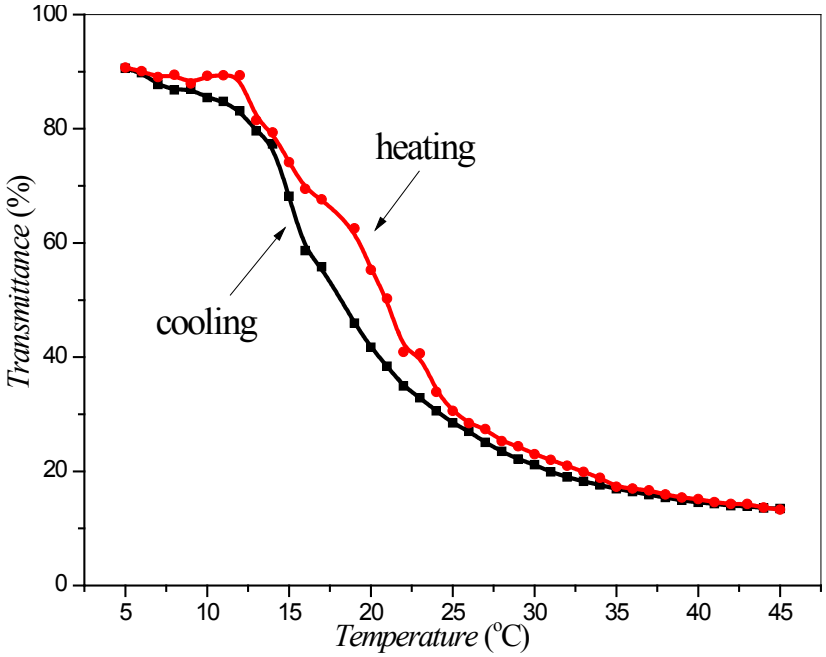


Fig. S9 Zeta potential and electromobility anginst temperature of PEEA vesicles at a C_{ini} of 0.5 mg/mL.

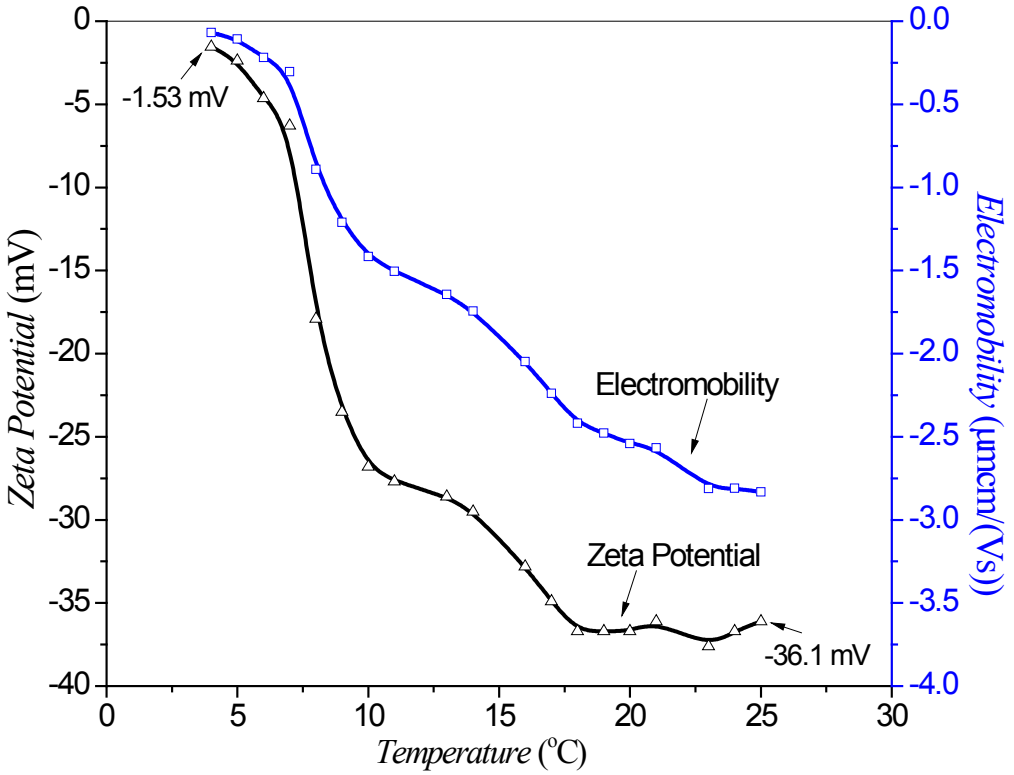


Fig. S10 Comulative release profile of DOX release experiment at pH 7.4. The control experiment was conducted by dialyzing DOX-tris buffer solution without PEEA vesicles. The contents of DOX are equivalent between experiments (a) and (b). The drug loading content and the drug loading efficiency are 5.6% and 22.2%, respectively. The calibration curve of the fluorescent intensity of DOX at various concentrations was conducted in 0.01 M tris buffer at pH 7.4 and 25 ^{o}C. 100% Cumulative DOX release is defined that the entire loaded drug in the vesicles is released, without any residual DOX in the vesicle or the dialysis tube.

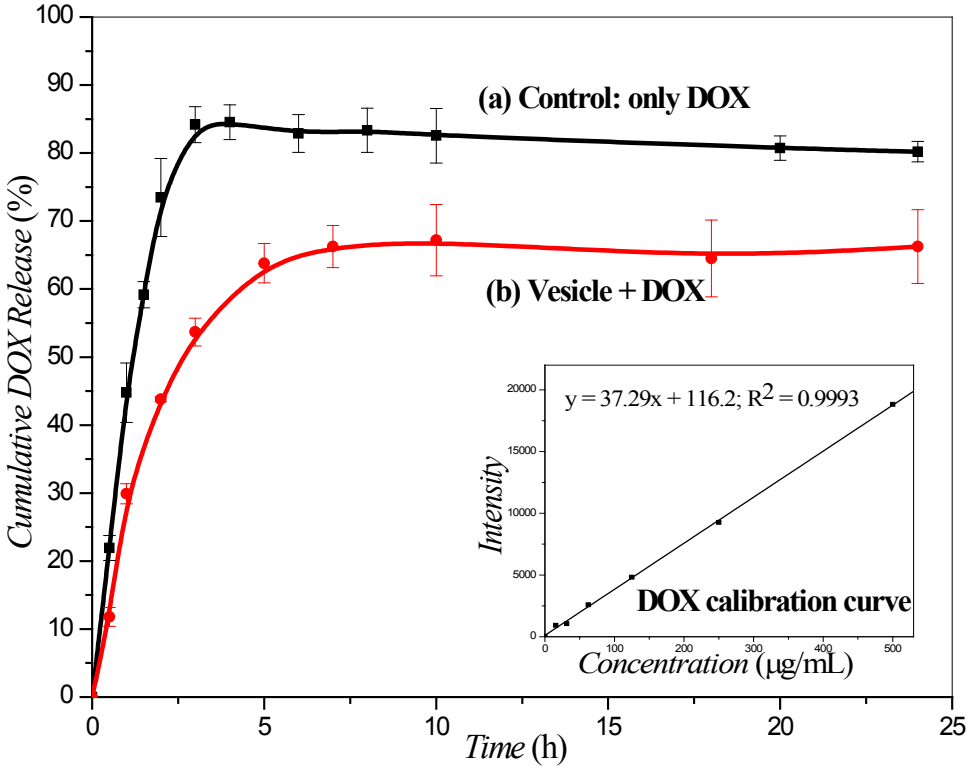
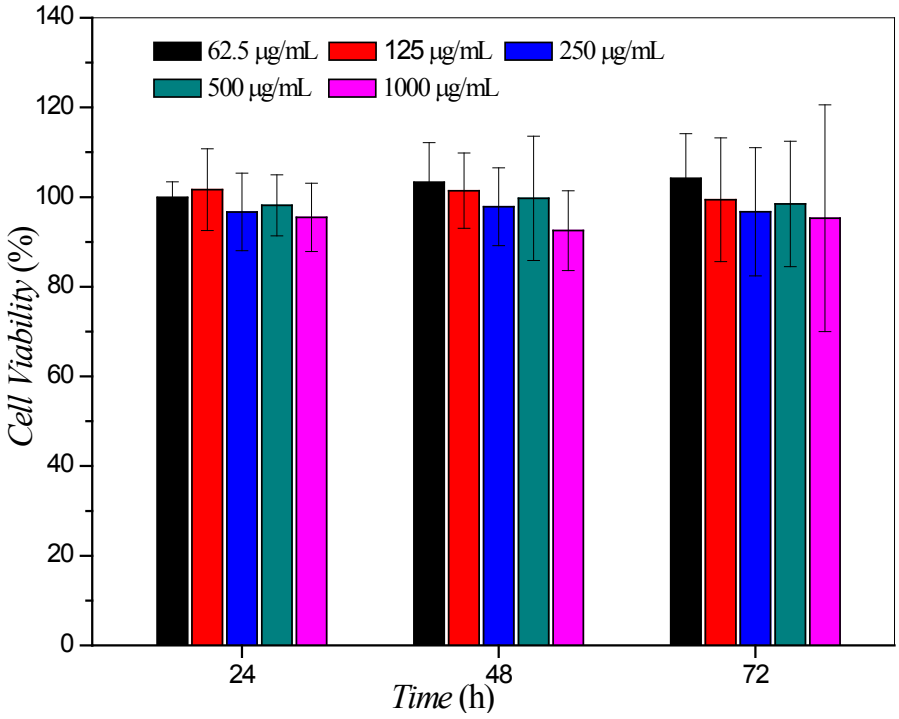


Fig. S11 Hela cell viability detected with CCK-8 assay. The cells were incubated with the homopolymer vesicles for 24 h, 48 h and 72 h. Results are presented as the mean ± SD in quintuplicate.



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2. G. Gaucher, M.-H. Dufresne, V. P. Sant, N. Kang, D. Maysinger and J.-C. Leroux, *Journal of Controlled Release*, 2005, **109**, 169-188.
3. J. Z. Du, L. Fan and Q. M. Liu, *Macromolecules*, 2012, **45**, 8275-8283.