Electronic Supplementary Information (ESI) for

Antibacterial vesicles by direct dissolution of block copolymer in water

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Experimental Section

Materials. 2-(*tert*-butylaminoethyl) methacrylate (TA) was purchased from J&K, dried over CaH_2 overnight, and distilled under reduced pressure prior to use. 2-(2-Methoxyethoxy)ethyl methacrylate (MEO_2MA) was purchased from J&K and was passed through columns filled with basic aluminum oxide. 2,2'-bipyridine (bpy), ethyl 2-bromoisobutyrate and 1,1,4,7,10,10-hexamethyltriethylenetetramine (HMTETA) were purchased from J&K and used as received. Toluene was dried by azeotropic distillation in the presence of sodium and benzophenone and then distilled under reduced pressure before use. Cu(I)Br was washed with acetic acid and ethanol alternatively for five times and then dried in vacuum. CH_2Cl_2, $CHCl_3$, THF and n-hexane was used as received.

The bacteria used in this work consisted of Gram-negative *E. coli* bacteria of ATCCC25922 strain and Gram-positive *S. aureus* bacterial of ATCC29253 strain. They were purchased from Nanjing BianZhen Biotechnology Co., Ltd. LB Agar and LB broth was purchased from Aladdin.

Characterization. THF GPC. GPC analysis was carried out with a Waters Alliance e2695 GPC analysis system with two styragel 5 μm columns (7.8×300 mm), using THF as eluent at a flow rate of 1.0 mL min^{-1} and 35 ^{o}C, PS calibration kit (purchased from TOSOH) as the calibration standard.

^{1}H NMR spectra were recorded using a Bruker AV 400 MHz spectrometer at ambient temperature using $CDCl_3$ as solvent.

Transmission electron microscopy (TEM) images were obtained using a JEM-2100 electron microscope equipped with a Gatan 1K ×1K digital camera operating at an acceleration voltage of 200 kV. To prepare TEM sample, 5 μL of the diluted acid aqueous copolymer solution (the initial

concentration was 0.5 mg/mL) at 37 ^{o}C (the heating rate was 3.5 ^{o}C/min) was placed on a preheated carbon-coated copper grid, and the water droplet was allowed to evaporate at 37 ^{o}C. The sample was stained by 1% phosphotungstic acid.

DLS studies were conducted using a Zetasizer Nano ZS90 instrument (Malvern Instruments) equipped with a multipurpose autotitrator (MPT-2) at a fixed scattering angle of 90^{o}. The data were processed by cumulants analysis of the experimental correlation function, and particle diameters were calculated from the computed diffusion coefficients using the Stokes-Einstein equation. Each reported measurement was conducted for three runs. The diameter of the vesicles and micelles in our research were measured using dynamic light scattering by quoting Stokes–Einstein equation ($D = kT/(3\pi\eta D_h)$).

$g_2(\tau)$, the auto-correlation functions can be expressed by following equation:

$$g_2(\tau) = \frac{I(t)I(t+\tau)}{I(t)^2} = 1 + B|(g_1(t))|$$

B is a constant, while τ is the measuring time. $g_1(t)$ can be derived by the following equation:

$$g_1(t) = e^{-\Gamma_1\tau + \frac{\Gamma_2}{2}\tau^2}$$

Γ_1 and Γ_2 stand for the first and second cumulant, respectively. The correlation decay was determined by the plots of Γ_1 vs. q^2. The processes of the test are diffusion-controlled, and D (= Γ_1/q^2) is related to D_h in Stokes–Einstein equation.

UV-vis study. The optical transmittance spectra for thermo-responsive properties were recorded at 500 nm wavelength using an UV–vis spectrophotometer (Shanghai Precision & Scientific Instrument Co., Ltd., UV759S).

Synthesis of $PMEO_2MA_{20}$-Br Macroinitiator by ATRP. A schlenk flask with a magnetic stir bar and a rubber septum was charged with ethyl 2-bromoisobutyrate (0.1070 g, 0.5376 mmol),

MEO_2MA (2.021 g, 10.75 mmol), CuBr (0.0783 g, 0.5376 mmol), bipyridine (0.1694 g, 1.075 mmol), and methanol (2.0 mL). The reaction system was degassed with three freeze-evacuate-thaw cycles and then the reaction was performed at 60 ^oC for 20 h under an argon atmosphere. Then the mixture was diluted with THF and passed through a neutral Al_2O_3 column to remove the copper catalyst. The product was collected by reprecipitation into cold n-hexane for three times and dried in a vacuum oven.

Synthesis of $PMEO_2MA_{20}$-*b*-PTA_{20} Diblock Copolymer by ATRP. $PMEO2MA_{20}$-Br (1.500 g, 0.3793 mmol), TA (1.475 g, 7.585 mmol), CuBr(0.0552 g, 0.3793 mmol), HMTETA(109.6 μL, 0.3793 mmol) and 2.0 mL toluene were added into a 100-mL round-bottom flask equipped with a magnetic stirrer. The reaction system was degassed with three freeze-evacuate-thaw cycles and then the reaction was performed at 70 ^oC for 24 h under an argon atmosphere. Then the mixture was diluted with THF and passed through a neutral Al_2O_3 column to remove the copper catalyst. The product was collected by reprecipitation into cold n-hexane for three times and dried in a vacuum oven.

Self-Assembly of $PMEO_2MA_{20}$-*b*-PTA_{20} Block Copolymer. $PMEO_2MA_{20}$-*b*-PTA_{20} copolymer (5.0 mg) was directly dissolved in water at pH 1.22 (10 mL). Increasing the solution temperature to 37 ^oC led to the formation of vesicles.

Antibacterial test: Copolymer acid solution of 0.50 mg/mL was prepared and heated to 37 ^oC with the heating rate of 3.5 $^oC\ min^{-1}$ to obtain vesicles. Then the pH was adjusted to 7.4 with aqueous NaOH solution. Various polymer vesicle solutions with concentrations of 0.25, 0.10 and 0.05 mg/mL were prepared by the serial dilution of the 0.50 mg/mL vesicle solution at 37 ^oC and pH 7.4. Then 100 μL of each vesicle solution were evenly spread on the surface of glass sheets

with the area of 1.0 cm^2. The water was allowed to evaporate slowly at 37 ^oC. Bacteria cells were cultured in LB overnight and 1.8 mL suspension was centrifuged and washed by physiological saline for three times. Then the bacterial suspension was diluted to 1.8 mL with physiological saline. Subesquently, 10 μL bacterial suspension was added to the surface of each glass sheet, which was then put flat into centrifuge tubes. The glass sheets were incubated at a relative humidity of not less than 90% for 2 h at 37 ^oC. After the incubation, 1.8 mL of physiological saline were added to the tube to dilute the survived bacterial with slight shaking. The survived bacterial solution was then diluted to different concentrations (10-fold). After 100 μL of each solution was placed in LB Agar. Then the plate was incubated for 1-2 days and 37 ^oC and the colony forming units were counted. Each concentration was incubated in three plates.

Critical aggregation concentration (CAC). The CAC of the $PMEO_2MA_{20}$-*b*-PTA_{20} triblock copolymer was determined by UV-vis spectroscopy using pyrene as a hydrophobic probe. 2.0 mg of pyrene was dissolved in 10 mL of acetone and 10 μL of the pyrene solution was added into each cuvette. The acetone was allowed to evaporate at room temperature. Then 4.0 mL of aqueous $PMEO_2MA_{20}$-*b*-PTA_{20} acidic solution ranging from 0.85 μg mL^{-1} to 250 μg mL^{-1} were added into the pyrene-containing cuvette separately, resulting in a theoretically maximum pyrene concentration of 2.0 μM in water. The solutions were kept at room temperature and equilibrated for several hours. Then the temperature of the solutions was increased to 37 ^oC gradually before UV measurements. The spectra were recorded in the 200–400 nm wavelength range. The obvious absorption peaks for pyrene were at 242, 272, 320 and 336 nm and the CAC was estimated by extrapolating the absorbance at 242 nm at various polymer concentrations. See Fig. S7 for the CAC value.

Calculation of the degrees of polymerization of block copolymers.

Table S1. The areas of different peaks and the degrees of polymerization of different copolymers

Spectrum	Polymer	a_{e+c+a}	a_i	a_b	a_m	a_{j+d}	x	y
Fig. S1	1	10	8.72	0.29			20	
Fig. S2	2		60		40.5	80.56		20

In Table S1, a_{e+c+a}, a_i, a_b,a_{j+d} and a_m are the areas of peaks e+c+a ($PMEO_2MA$, 3x+9 H), i ($PMEO_2MA$, 3x H), b ($PMEO_2MA$, 2 H), j+d ($PMEO_2MA$ + PTA, 2x + 2y H) and m (PTA, 2x H) in Fig. S1 and Fig. S2. x and y are the degrees of polymerization of MEO_2MA and TA. Polymers 1 and 2 represent $P(MEO_2MA)_x$-Br macroinitiator and $P(MEO_2MA)_x$-*b*-PTA_y diblock copolymer.

Polymer 1: We set that the integration area of peak e+c+a is 10. According to seperated peaks i ($PMEO_2MA$) and b ($PMEO_2MA$), and overlapped e+c+a ($PMEO_2MA$):

Block length of $PMEO_2MA$ (comparing peak i with peak e+c+a):

$$\frac{3x}{8.72} = \frac{3x+9}{10} \quad \rightarrow \quad x = 20.4 \approx 20$$

Block length of $PMEO_2MA$ (comparing peak b with peak e+c+a):

$$\frac{2}{0.29} = \frac{3x+9}{10} \quad \rightarrow \quad x = 19.9 \approx 20$$

Polymer 2: We set that the area of peak i is 60 according to x = 20 in polymer 1, which corresponds to the amount of H in $PMEO_2MA_{20}$ (30 × 2 = 60). According to seperated peaks i ($PMEO_2MA$) and m (PTA), and overlapped j+d ($PMEO_2MA$ + PTA):

Block length of PTA (comparing peak m with peak i):

$$y = \frac{40.5}{60} \times \frac{20 \times 3}{2} = 20.25 \approx 20$$

Block length of PTA (comparing peak j + d with peak i):

$$y = \frac{80.56 - 20 \times 2}{60} \times \frac{20 \times 3}{2} = 20.28 \approx 20$$

Fig. S1 ^{1}H NMR spectrum of $PMEO_2MA_{20}$-Br macroinitiator with integrals in $CDCl_3$.

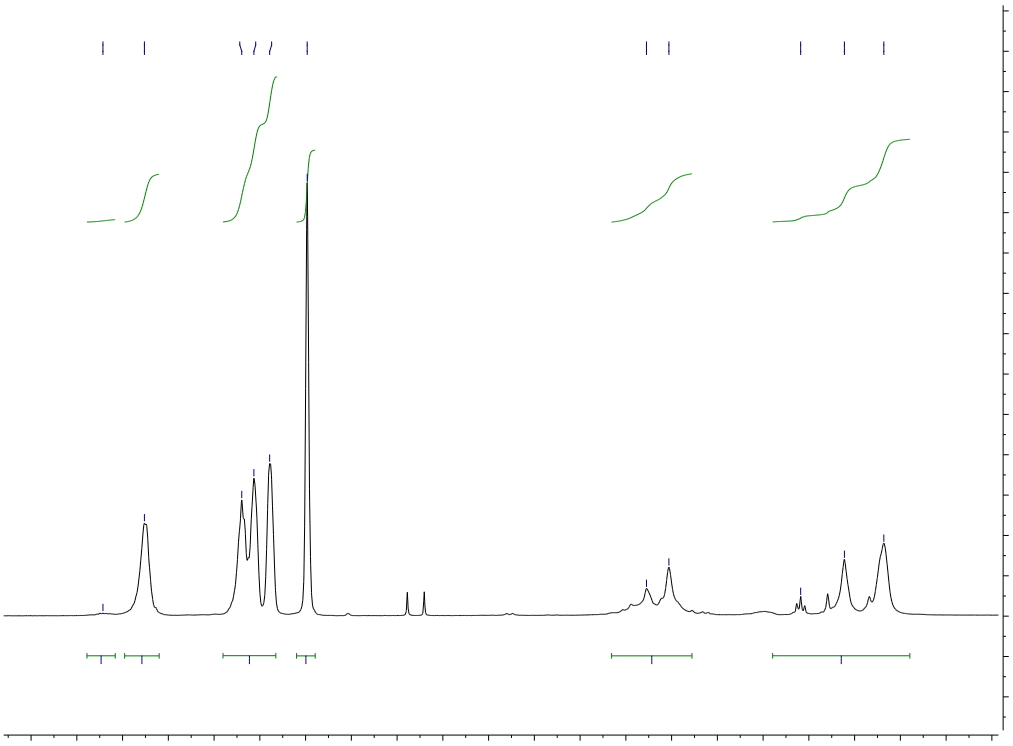


Fig. S2 ^{1}H NMR spectrum of $PMEO_2MA_{20}$-*b*-PTA_{20} diblock copolymer with integrals in $CDCl_3$.

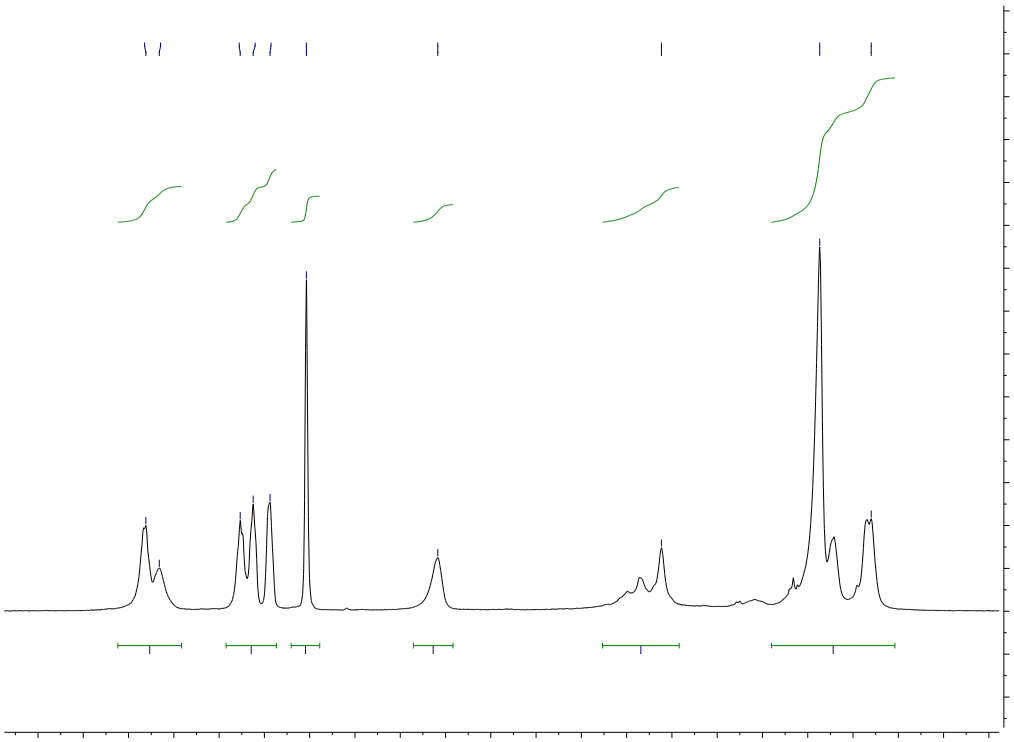


Fig. S3 GPC traces of $PMEO_2MA_{20}$-Br macroinitiator and $PMEO_2MA_{20}$-*b*-PTA_{20} diblock copolymer in THF. The molecular weights calculated from ^1H NMR spectra are 3955 and 7655 for $PMEO_2MA_{20}$-Br and $PMEO_2MA_{20}$-*b*-PTA_{20}, respectively.

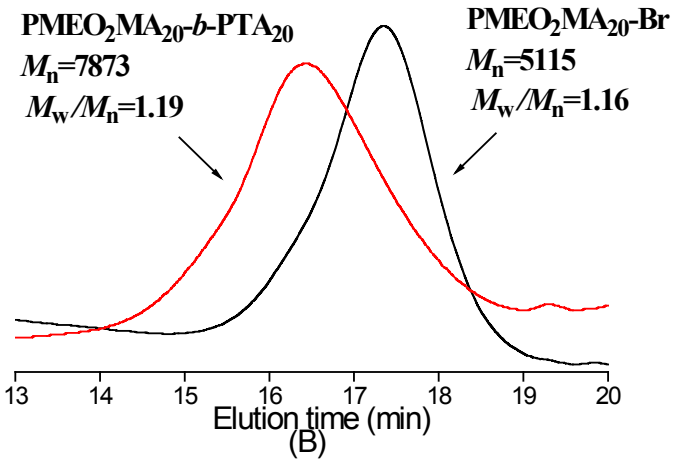


Fig. S4 The effect of heating rate on the size of $PMEO_2MA_{20}$-*b*-PTA_{20} vesicles obtained from the temperature increase of 0.5 mg mL^{-1} acidic polymer solution from 20 °C to 37 °C.

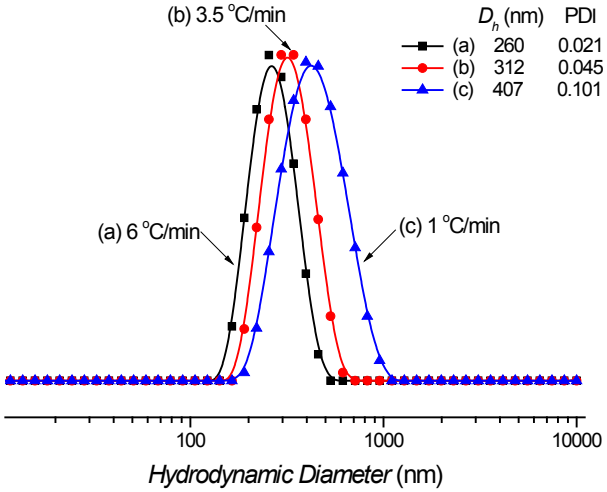


Fig. S5 (A) DLS result (by number) of vesicles prepared from the temperature increase of the acidic polymer solution from 20 °C to 37 °C with a heating rate of 3.5 °C/min; (B) DLS studies of the (A) vesicle solution with pH adjusted to 7.4 at 37 °C.

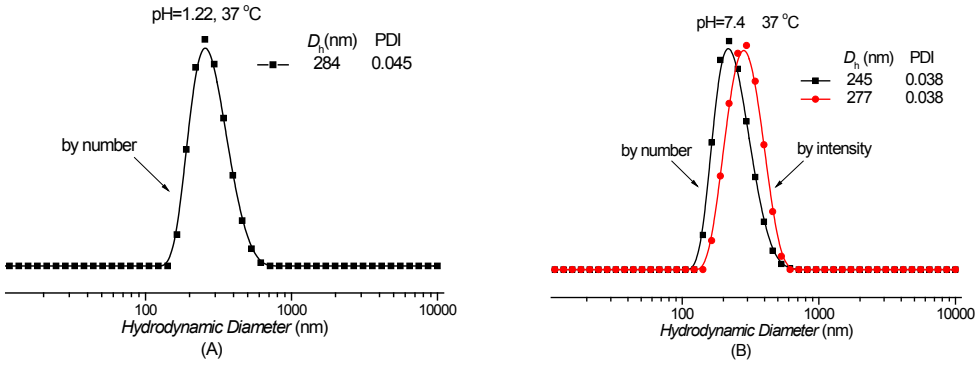
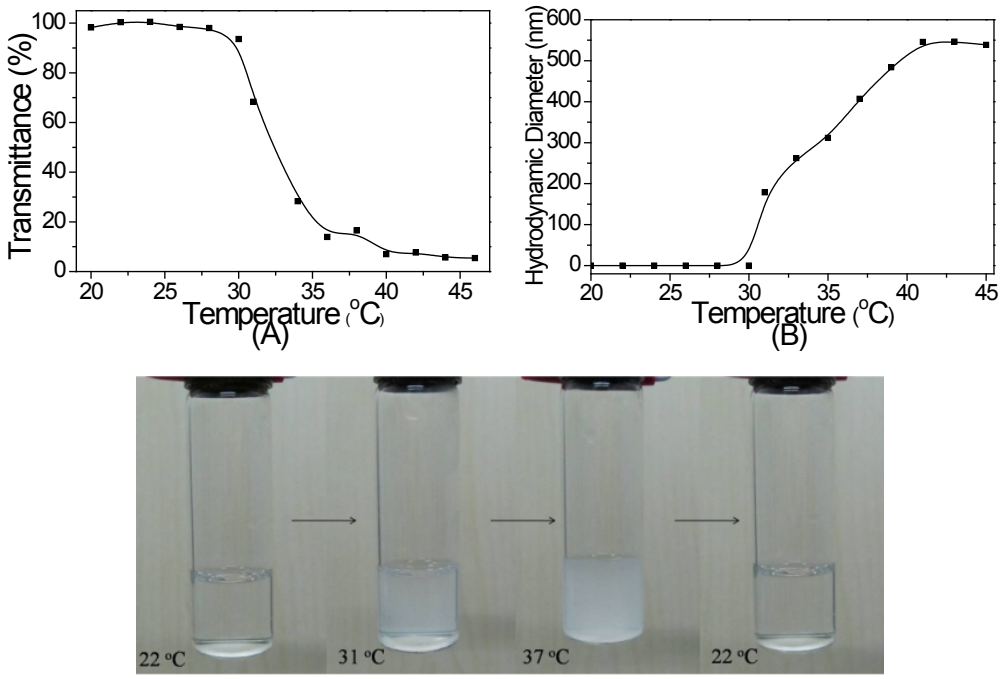


Fig. S6 Variation of (A) Optical transmittance and (B) Z-averaged hydrodynamic diameters as a function of temperature for aqueous $PMEO_2MA_{20}$-*b*-PTA_{20} copolymer solution at 0.5 mg/mL and pH 1.22. The heating rate is 1.0 °C/min. The Digital photographs (C) illustrate the reversible changes of the polymer solution turbidity with increasing and decreasing temperatures.



(C)

Fig. S7 UV absorbance of pyrene at 242 nm as a function of the concentration of $PMEO_2MA_{20}$-*b*-PTA_{20} in acidic water at 37 °C. This experiment confirmed the CAC is 39 μg/mL.