Electronic Supplementary Information (ESI) for

Ultrafine silver nanoparticles with excellent antibacterial efficacy by handover of vesicle templating to micelle stabilization

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Experimental Section

Materials and methods

Polyethylene glycol monomethylether (MeO-PEO-OH; M_n = 1900) was purchased from Alfa Aesar and dried azeotropically by using anhydrous toluene to remove traces of water. *t*-Butyl acrylate (*t*BA) monomers were obtained from Sinopharm Chemical Reagent CO., Ltd and Tokyo Chemical Industry CO., Ltd, respectively. A silica column was used to remove the inhibitor before use.

N,*N*,*N'*,*N''*,*N''*-pentamethyldiethylenetriamine (PMDETA, 98%) and triethylamine were purchased from Aladdin Chemistry, CO. Tetrahydrofuran (THF), 2-bromoisobutyryl bromide, copper(I) bromide (CuBr, 99.999%) and other reagents were used as received. Dialysis tubing with molecular weight cutoff from 8000 to 14000 was purchased from Sinopharm Chemical Reagent CO., Ltd (SCRC, Shanghai, China).

Characterization

DLS Studies. Zetasizer Nano series instrument (Malvern Instruments ZS 90) equipped with a multipurpose autotitrator (MPT-2) was used to conduct the studies. DLS studies of aqueous polymer vesicles were carried out at a fixed scattering angle of 90^o. Stokes-Einstein equation was used to calculate the hydrodynamic diameters of polymer vesicles. Each reported measurement was conducted three runs. The hydrodynamic diameter of the vesicles were measured using DLS 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.

TEM images were obtained using a JEM-2100 electron microscope operating at an acceleration voltage of 200 kV. To prepare TEM samples, 10 μL of diluted aqueous vesicle solution at 1.0 mg mL^{-1} was placed on a carbon-coated copper grid. 1.0% of phosphotungstic acid (PTA) was used as stain. The water droplet was removed by evaporation under ambient conditions.

AFM was employed to verify the hollow structure of polymer vesicles. The vesicle solution was diluted at ambient temperature to 50 μg/mL and dropped (10 μL) onto the silica wafer (1×1 cm^2) and dried at room temperature. The silica wafer was washed with acetone for 4 times before sample preparing. The observation was conducted on a Seiko (SPA-300HV) instrument operating in a tapping mode at 200-400 kHz drive frequency.

The UV−vis absorption spectra of aqueous polymer solution and silver nanoparticles were acquired using a UV-759S spectra (UV-759S, Q/YXL270, Shanghai Precision & Scientific Instrument Co., Ltd) to monitor the UV absorption changing of the solution.

The fluorescence study was carried out at the emission wavelength of 372.1 nm and excitation wavelength of 334 nm using pyrene as the fluorescence probe. The fluorescence spectroscopy is purchased from Thermo Fisher (LF-1202002).

Self-Assembly of PEO_{43}-*b*-P(*t*BA_{15}-*stat*-AA_{50}) Block Copolymer into Vesicles

PEO_{43}-*b*-P(*t*BA_{15}-*stat*-AA_{50}) copolymer (30.0 mg) was firstly dissolved in THF, then water was added at a rate of approximately one drop every 2 s with vigorous stirring. After 2 h of stirring, the organic solvent was removed via dialysis against water for 48 h.

Preparation of silver nanoparticles by handover of vesicle templating to micelle stabilization

The aqueous PEO_{43}-*b*-P(*t*BA_{15}-*stat*-AA_{50}) vesicle solution (1.0 mg mL^{-1}; 10 mL) was mixed with $AgNO_3$ solution (1.0 mg mL^{-1}; 1.0 mL). After gently stirring for 30 min in the dark at room temperature, the solid $NaBH_4$ (3.0 mg) was then quickly added to the vesicle solution. The solution immediately became yellow after adding $NaBH_4$. After 4 h of reduction, the solution was further purified by dialysis against water for 20 h and the final pH is ~ 7.4.

Antibacterial Test against *Escherichia coli* and *Staphylococcus aureus*

The inhibitory experiments were carried out with Gram-negative bacteria *E. coli* and Gram-positive bacteria *S. aureus*. Firstly, the silver nanoparticles with initial nano-silver concentrations at 67.6 μg mL^{-1} were sterilized under UV light for 30 min. Several concentrations of silver nanoparticles (16.9, 8.45, 4.23, and 2.11 μg mL^{-1} for silver nanoparticles) were added to the test tube with the bacterial suspension (10 μL of 2×10^7 CFU mL^{-1}) against *E. coli*. The MICs of the nano-silver were measured using a Luria–Bertani (LB) medium broth micro-dilution method at the concentration of which no bacteria growth was observed in the test tube after it incubated for 20 h at 37°C.

Fig. S1. Size distribution of polymer vesicles determined by DLS by intensity at 1.0 mg mL^{-1}, pH 6.3 and 25°C.

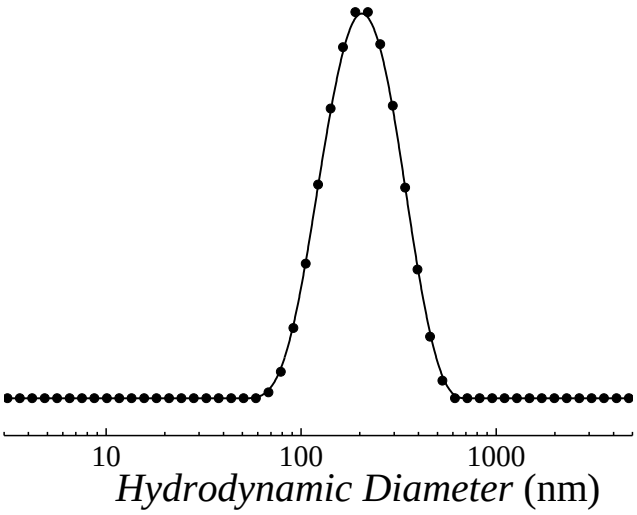

Fig. S2. CVC and CMC measurements of the PEO_{43}-*b*-P(*t*BA_{15}-*stat*-AA_{50}) block copolymer vesicles at pH 6.3 (A) and block copolymer micelles at pH 10.0 (B). Fluorescence emission spectrum of pyrene at 383.1 nm is a function of the copolymer concentration in pure water at 25 ^{o}C. This experiment confirmed the CVC and the CMC of the copolymer at pH 6.3 and pH 10.0 are 38.9 μg mL^{-1} and 24.5 μg mL^{-1}, respectively.

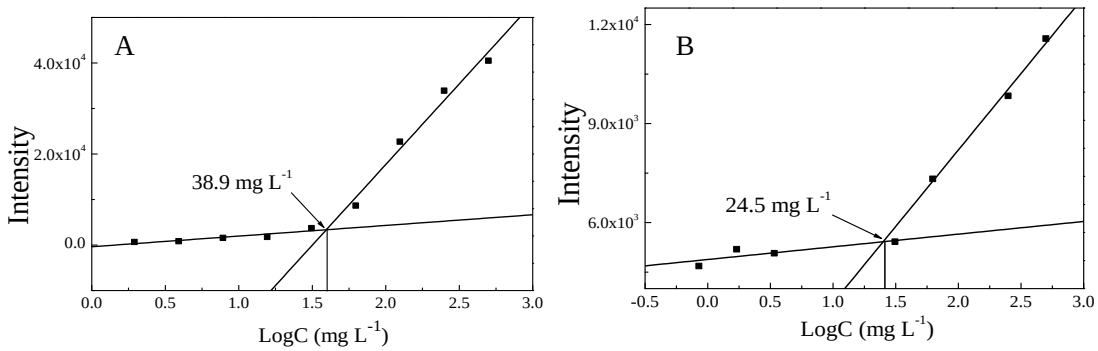

Fig. S3. Hydrodynamic diameter of polymer vesicles and micelles at various pH values determined by DLS (by number) at 25 ^{o}C. The switching point of the transition between vesicles and micelles is around pH 7.1.

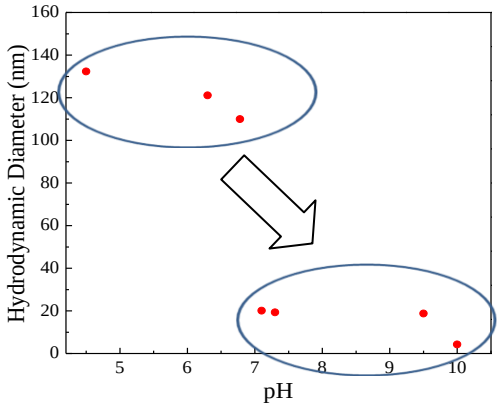

Fig. S4. (A) The derived count rate of polymer vesicles (< pH 7.1) and micelles (> pH 7.1) at various pH determined by DLS at 25 ^{o}C; (B) photos of the vesicle and micelle solution at different pH.

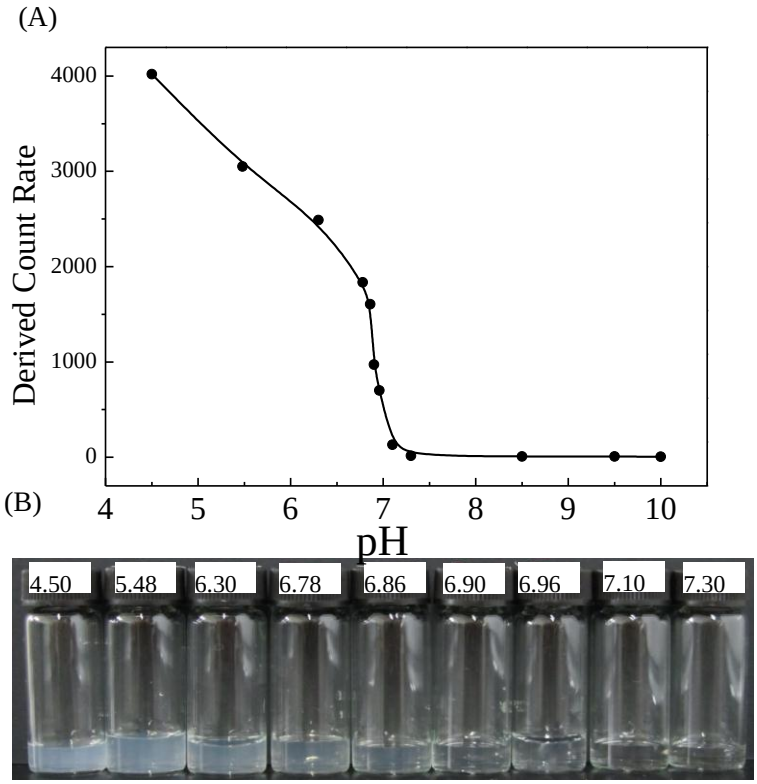

Fig. S5. TEM image of ultrafine silver nanoparticles.

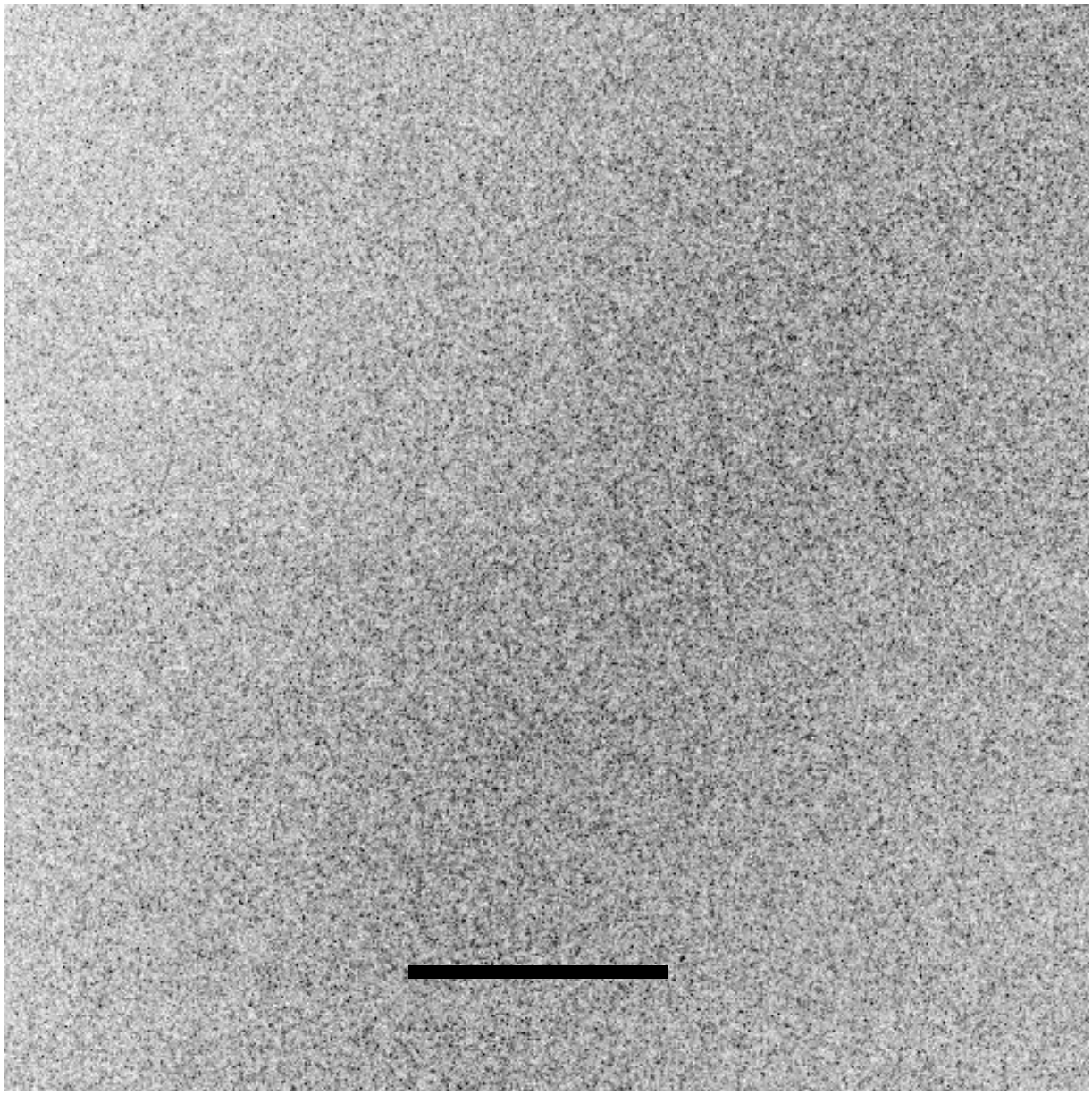

Table S1. MIC test of polymer vesicle template without silver nanoparticles against *E. coli* and *S. aureus*.[a)]

polymers		
C_p/ μg mL^{-1}	*E. coli*	*S. aureus*
400	+	+
200	+	+
100	+	+
50	+	+

[a)] "+" for bacteria growth, "–" for no bacteria growth; C_p: concentration of polymer.