Supplementary Material

Enhanced bactericidal toxicity of silver nanoparticles by antibiotic gentamicin

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1. Joint antibacterial property of Ag-PVP NPs and antibiotics

Table S1 The minimal inhibition concentration (MIC) of Ag-PVP NPs, ampicillin and penicillin G, and fractional inhibitory concentration (FIC)[a] of combinations of ampicillin/penicillin G and Ag-PVP NPs against *E. coli* and *S. aureus*.

Bacterial strain	MIC (μg mL^{-1})			FIC index (interpretation)	
	Ag-PVP NPs	ampicillin	penicillin G	ampicillin	penicillin G
E. coli (DH5-α)	100	0.5	2	2.10 (A)	1.10 (AD)
E. coli (ATCC 25922)	100	0.5	2	2.00 (A)	1.00 (AD)
S.aureus (ATCC 25923)	100	0.125	2	2.10 (A)	1.10 (AD)

[a] The abbreviations for interpretations: A, antagonism; AD, additivity; I, indifference; S, synergy.

2. Determination of silver dissolved from Ag-PVP NPs by the UV-vis method

The surface plasmon resonance of Ag-PVP NPs is available in a certain range of diameter (four to a few hundred nanometers). Ag-PVP NPs are regarded as nanocluster rather than nanoparticles when their diameter is less than 2 nm, which exhibit weak SPR spectra. The UV-Vis method could distinguish Ag NPs from dissolved silver and other Ag-containing particles, such as AgCl, by detecting the characteristic absorption intensity of Ag-PVP NPs (Zook et al., Anal. Bioanal. Chem. 401, 1993-2002). The UV-Vis spectra of Ag-PVP NPs of different concentrations are presented in Fig. S1. It is observed that only when the concentration of Ag-PVP NPs is below 0.2 μg mL^{-1} (1.6% of 12.5 μg mL^{-1} Ag-PVP NPs used in this study), the UV-vis absorption of Ag-PVP NPs is not visible.

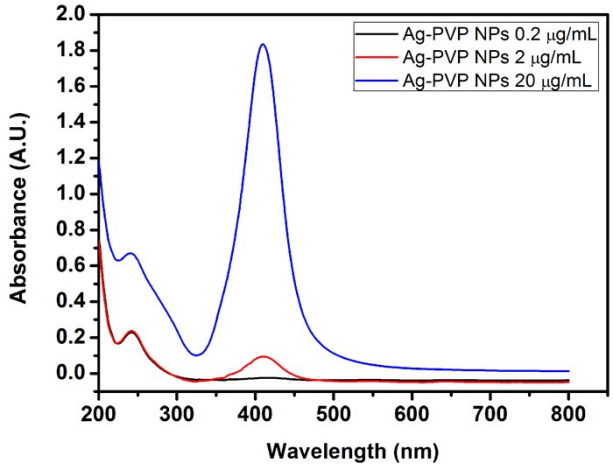

Figure S1. UV-vis spectra of Ag-PVP NPs of different concentrations.

The amount of dissolved Ag was determined by scanning the absorption spectra of the Ag-PVP NP suspension from 200 to 800 nm using a microplate reader (Thermo, Varioskan Flash, USA) (Fig. S2). Briefly, 100 μL of suspension of 12.5 μg mL^{-1} Ag-PVP NPs either in ultrapure water or water containing different concentrations of gentamicin was pipetted into a well of a 96-well microplate. Then the plate was incubated in a rotary shaker at 150 rpm at 37 ºC. The UV-vis spectra of the sample at certain incubation time points were scanned. For calculation, the difference in peak values between 0 and 12 h are used to quantify the decrease in Ag-PVP concentration. The absorption values of water or gentamicin were subtracted from those of Ag-PVP

NP suspensions, respectively. Resulting percentage of released silver ions are plotted versus incubation time. In addition, wider peak reflects a wider size distribution of Ag PVP NPs in suspension.

Quite similar results verify the reliability of the two methods (ICP-MS and UV-vis method) since if the nanoparticles are too small to be effectively separated from solution using centrifugation, as a result, the concentration values for the percent of dissolved silver for Ag-PVP NPs using ICP-MS method (which relies upon centrifugation for separation) will be anomalously high. Therefore, the values obtained using either method is reliable in this study.

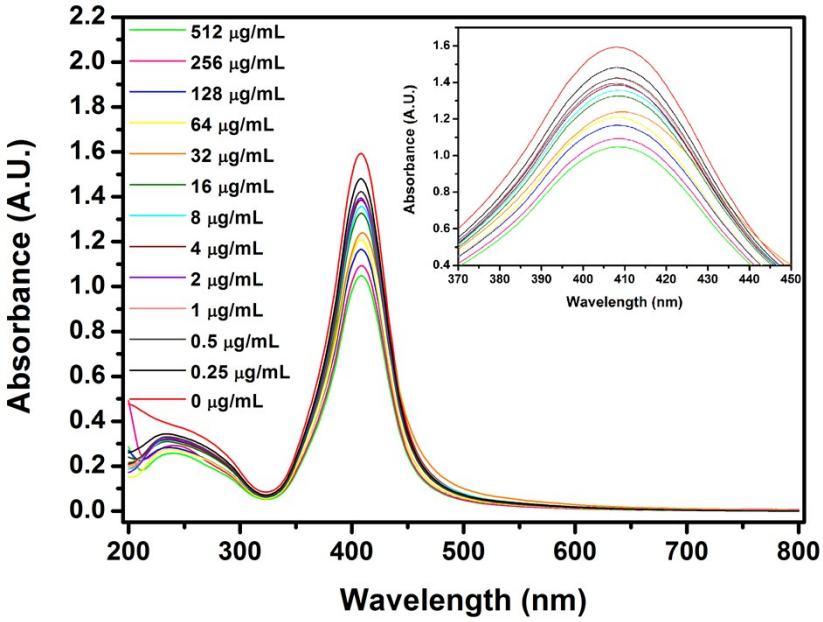

Figure S2. UV-vis spectra of 12.5 μg mL^{-1} Ag-PVP NPs in ultrapure water after adding different concentrations of gentamicin for 1 h. Insert is the partial enlarged details.

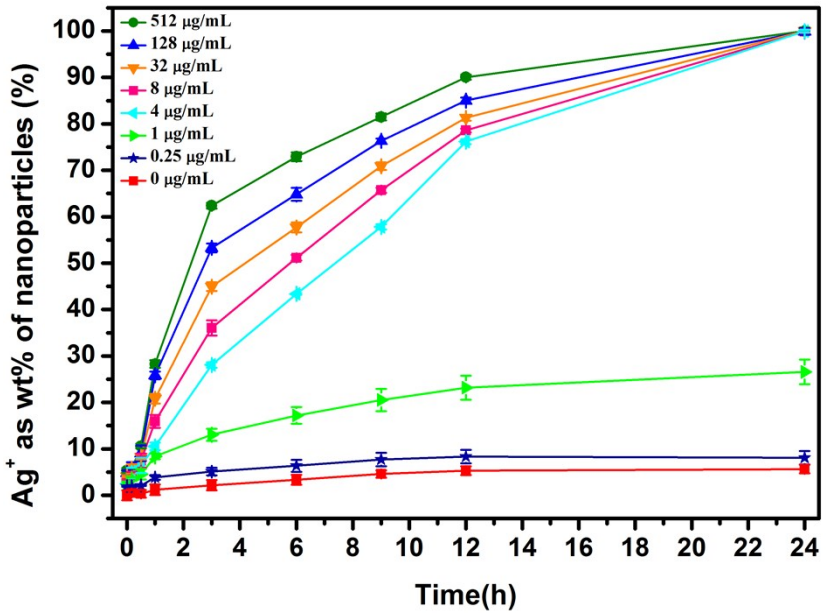

Figure S3. Dissolution of 12.5 μg mL^{-1} Ag-PVP NPs in ultrapure water containing gentamicin of different concentrations. Ag-PVP NPs suspended in deionized water were shaken on the platform of a shaker at 37° C for 12 h. Dissolved Ag is calculated from the differences in maximum absorbance values of UV-vis spectrum of Ag-PVP NPs at certain time intervals.

3. TEM investigation of Ag-PVP NPs in MH medium in the presence of gentamicin

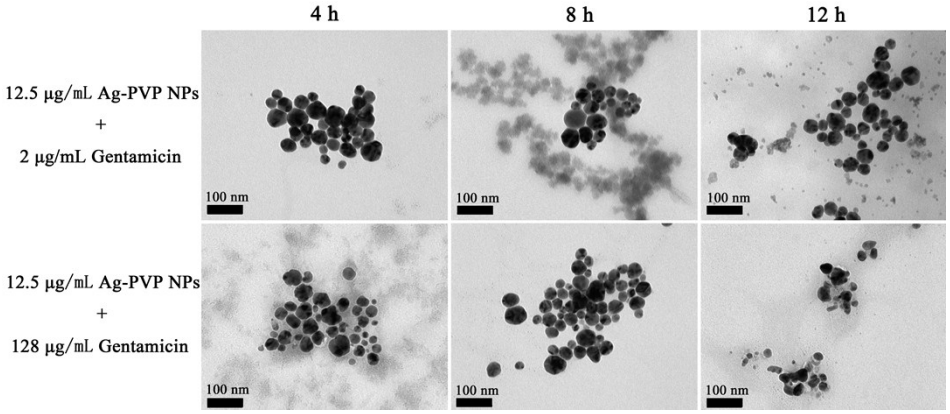

Figure S4. TEM images of 12.5 μg mL^{-1} Ag-PVP NPs after treated with 2 μg mL^{-1} or 128 μg mL^{-1} gentamicin in MH medium at 37 °C for 12 h.

4. Gaussian fitting curve of antibacterial property of Ag ions in the presence of

gentamicin

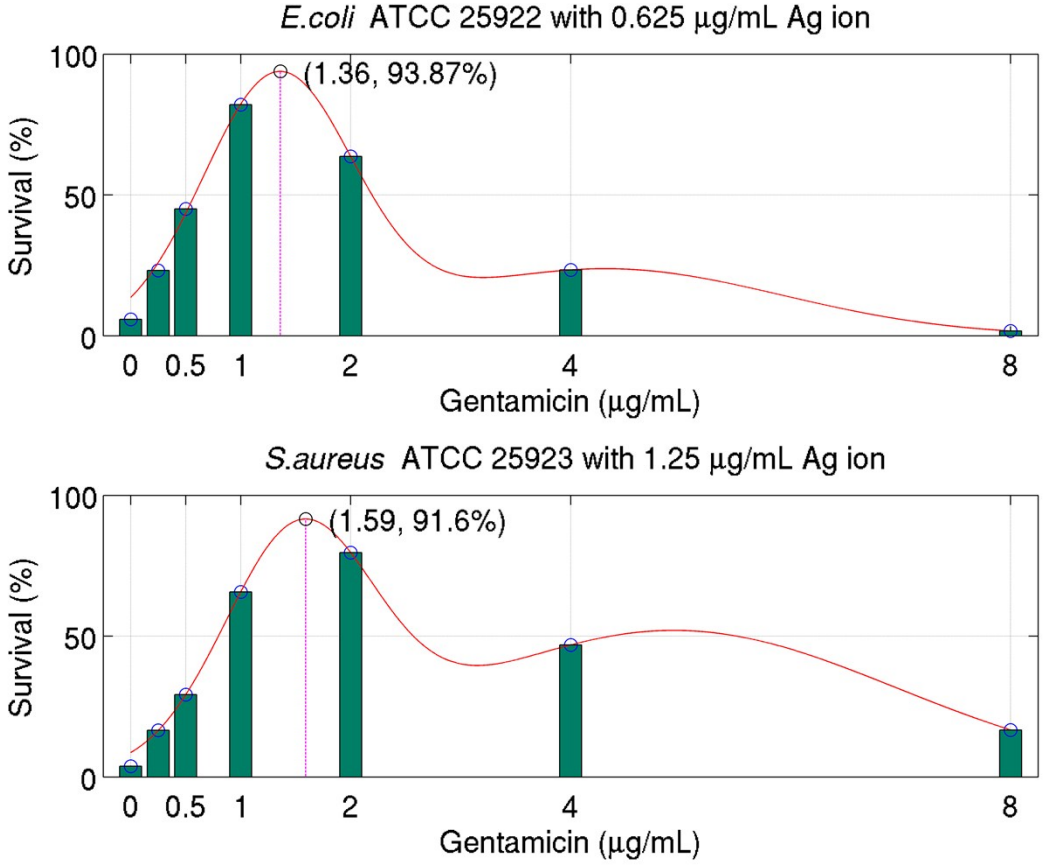

Figure S5. Antibacterial property of Ag ions in the presence of gentamicin. *E. coli* ATCC 25922 (upper) and *S. aureus* ATCC 25923 (bottom) were exposed to Ag ion and gentamicin of different concentrations for 12 h at 37 °C. The red line is the Gaussian fitting curve. The peak points marked by black circles in the figure denote the maximum bacterial survival rate, that is, the minimum antibacterial efficiency against *E. coli* and *S. aureus*, receptively.

5. Observation of the interaction between silver ion and gentamicin by the electrochemical method

Electrochemical experiments were performed using an electrochemical workstation (CHI604D, CH Instruments, Inc., TX, USA) in a conventional three-electrode arrangement, equipped with a glassy carbon working electrode, a platinum counter electrode and a calomel reference electrode. Potentials were quoted versus the calomel reference. Glassy carbon electrodes (3.0 mm diameter) were polished using 1.0 and 0.5 µm alumina powder and cleaned in an ultrasonic bath before use. All electrochemical measurements were conducted at room temperature of about 20 °C.

The mixture solutions of silver nitrate (0.8 mM) and gentamicin (from 0 mM to 30 mM) in 0.2 M $NaNO_3$ electrolyte (pH=7) were freshly prepared for cyclic voltammetry measurement. Voltammetric measurements were conducted with a scan rate of 100 $mV \cdot S^{-1}$ and scan range from 0.8 V to -0.4 V.

When only silver nitrite existed in electrolyte, the anodic peak at +0.46 V should correspond to the oxidation of silver metal and the corresponding peak current. The cathodic peak at +0.24 V corresponded to the reduction of the oxidative silver species in solution (Song et al., J. Electroanal. Chem. 2001, 498, 161-170). The cathodic process also produced a broad tail extending to zero potential and the reduction peak current was much smaller than the oxidation peak current.

In the presence of gentamicin, both the anodic and cathodic peaks were shifted and reduced in a gentamicin concentration-dependent manner, indicating the decrease of the concentration of free silver ions. Thus confirms the interaction between gentamicin and silver ion.

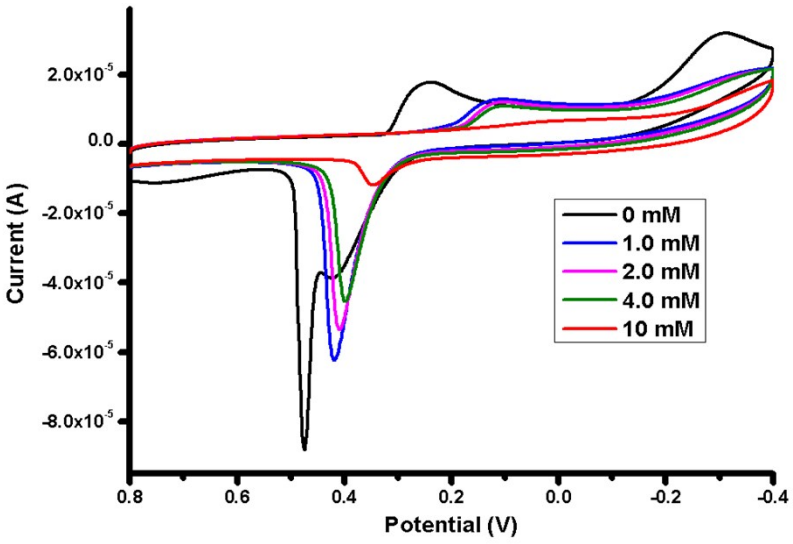

Figure S6. Cyclic voltammograms of silver species (0.8 mM) with gentamicin varied from 0 mM to 10 mM. Supporting electrolyte: $NaNO_3$ solution at pH 7.