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The Supporting Information for High-Efficient Nanomedicine from Cationic Antimicrobial Peptides Protected Ag Nanoclusters

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Materials and reagents

Bactenecin (Opep) was purchased from Aladdin (Shanghai, China). Dpep (CCLLLRRRRRR) was synthesized from Sangon Biotechnology Co., Ltd. (Shanghai, China). $NaBH_4$ and $AgNO_3$ were bought from Sinopharm Chemical Regent Co., Ltd (Shanghai, China). NaOH and CH_3COOH were obtained from Beijing Chemical Works (Beijing, China). Commercial Ag NPs of 30 nm were obtained from Yunfu Nanotechnology Co., Ltd. (Shanghai, China). *E. coli* K-12 strain with chloramphenicol-resistance was purchased from Yale University (New Haven, USA). Bacterial used culture medium MH broth was from Hope Biotechnology Co., Ltd. (Qingdao, China). Cell culture medium Dulbecco's modified eagle medium (DMEM) and fetal bovine serum (FBS) were from Gibco (Grand Land, New York, USA). Cell counting kit-8 (cck-8) was from Dojindo Molecular Technologies (Shanghai, China). All of the glass made vessels and magnetons were washed with a bath of aqua regia, rinsed with ethanol and then ultrapure water. Sterile ultrapure water (18.2 MΩ •cm^{-1}) from Millipore system was used throughout the experiment.

Apparatus and characterizations

UV-vis absorption spectra and fluorescence spectra were recorded on Cary 60 UV-vis and Cary Eclipse fluorescence spectrophotometer (Agilent Technologies, USA), respectively. Transmission electron microscopy (TEM) and high resolution transmission electron microscopy (HRTEM) were obtained by a JEM-2100F high resolution transmission electron microscope (Netherlands) which was operated at 200 kV. A quadrupole-time of flight (Q-TOF) SYNAPT G2 high definition mass spectrometer (HDMS) (Waters Crop., Manchester, UK) was used to obtain chemical composition of Opep-Ag NPs. X-ray Photoelectron Spectroscopy (XPS) was obtained from an ESCALAB-MKII X-ray photoelectron spectroscopy (VG Scientific, UK). Inductively coupled plasma mass spectrometry (ICP-MS) results were recorded by using a Thermo Scientific iCAP6300. OD_{600} was measured on a YuanXi UV5800 ultraviolet-visible spectrophotometer (Shanghai, China) to determine the concentration of the bacteria solution.

ROS level determination

ROS generation level was determined by the ROS Assay Kit. DCFH-DA in the toolbox is non-fluorescent and is free to pass through the membrane until hydrolyzed by intracellular esterase to produce DCFH. DCFH accumulate in the cells and become green fluorescent after oxidized into DCF by the intracellular ROS. So it's easy to detect the ROS level generated by Dpep-Ag NCs and other counterparts according to their fluorescence intensity.

Minimum Bactericidal Concentrations (MBCs) determination towards *E. coli*

We also determined the MBCs of the Dpep-Ag NCs, Opep-Ag NPs, BSA-Ag NCs and Ag NPs (30 nm) towards *E. coli*, respectively. The number of CFUs was counted after subculturing MIC and three more concentrated wells dilutions onto the agar plates. The MBC is the lowest concentration that demonstrates a reduction of 99.9% in CFU/mL compared to the MIC dilution.

Cell culture and cytotoxicity assay

HDF cells were cultured in DMEM with 10% FBS supplemented for 2 days. Then they were digested with trypsin enzyme (0.25% EDTA added), washed and transferred into a 96-wells plate with 10^4 cells dispersed in fresh culture medium each well. After cultivation for 12 h, diluted AMP-Ag NPs using the fresh culture medium at MIC were introduced to incubate the HDF cells. After incubation for 2 h at 37 ℃, the Dpep-Ag NCs and Opep-Ag NPs containing medium was removed and the samples were washed twice with sterile PBS. 10 μL cck-8 diluted in 90 μL DMEM was added into each well for further incubation to evaluate the cytotoxicity. Cell viability was determined by measuring absorbance at 450 nm using a microplate reader. The cells were cultured in humidified incubator at 37 ℃ and 5% CO_2 supplemented.

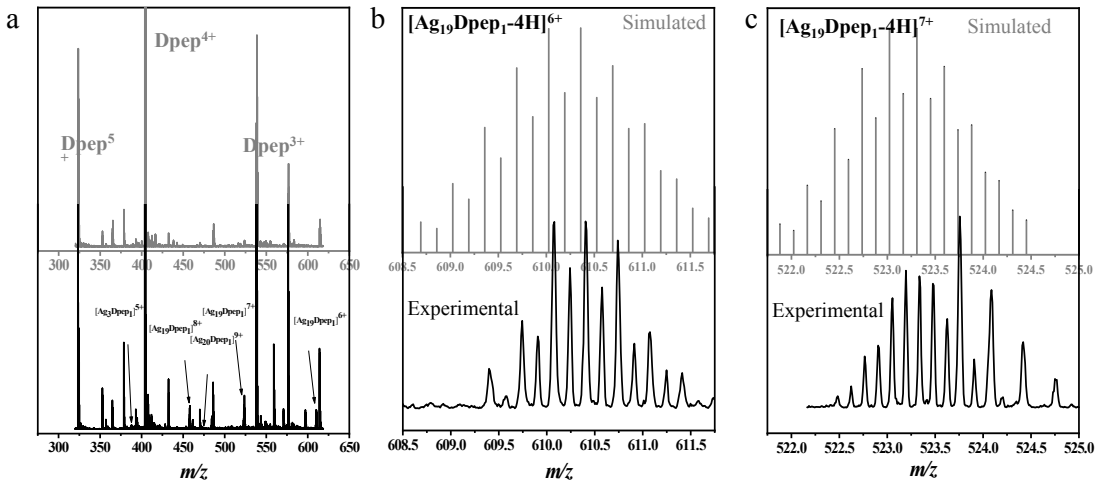

Figure S1 (a) The HD-MS spectra (collected in positive ion mode) of the pure Dpep (upper) and Dpep-Ag NCs (below), respectively; the +6 (b) and +7 (c) charged $[Ag_{19}Dpep_1\text{-}4H]$ NCs and its simulated spectra, respectively. The position of $[Ag_{19}Dpep_1\text{-}4H]^{7+}$ NCs have some degree overlay with that of triply charged [Dpep-COOH+4H]. The minor difference between the experimental isotope patterns with the simulated ones, especially at the higher m/z end, maybe caused by the instrumental error.

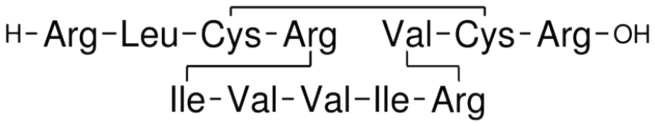

Figure S2 The sequence of Bactenecin (Opep, $C_{63}H_{118}N_{24}O_{13}S_2$, Mw=1485).

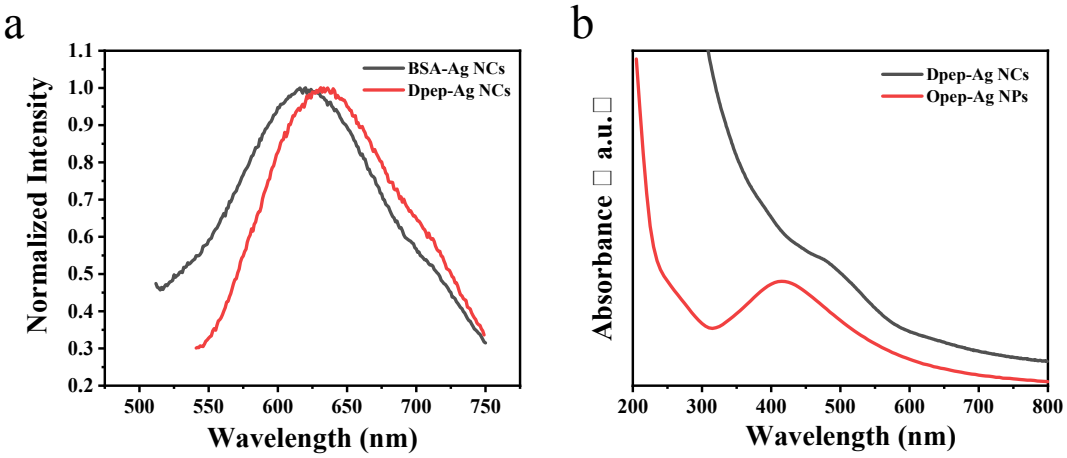

Figure S3 (a) The photoluminescence characterization of the prepared BSA-Ag NCs and Dpep-Ag NCs, respectively. (b) Optical absorbance spectra of the Dpep-Ag NCs and Opep-Ag NPs, respectively.

Table S1, the MBCs of Dpep-Ag NCs, Opep-Ag NPs, BSA-Ag NCs and Ag NPs (30 nm) towards *E. coli*, respectively.

	Dpep-Ag NCs	**Opep-Ag NPs**	**BSA-Ag NCs**	**Ag NPs (30 nm)**
MIC (μM)	6.5 μM	24 μM	120 μM	800 μM
MBC (μM)	52 μM	96 μM	480 μM	3200 μM

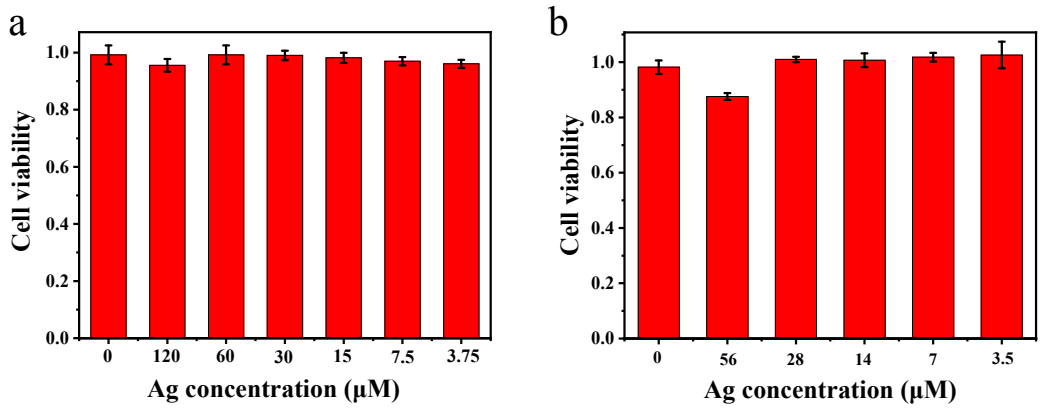

Figure S4 The HDF cell viability after treating Opep-Ag NPs and Dpep-Ag NCs, respectively.