Supporting Information

Chemicals and materials

Nanopure water (18.2 Meghom·cm; Millpore Co., USA) was used in all experiments and to prepare all buffers. N-cetyltrimethylammonium bromide (CTAB), 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and aminopropyltriethoxysilane (APTES) were purchased from Alfa Aesar. Fuorescein isothiocyanate (FITC), tetraethylorthosilicate (TEOS), D-Propargylglycine (ethynyl-D-alanine) and 4,7,10-Trioxa-1,13-tridecanediamine (TTDDA) were obtained from Sigma-Aldrich. L-Propargylglycine (ethynyl-L-alanine, CAS:23235-01-0) and Bromoacetic acid were purchased from J&K Scientific Ltd.. fluorescein (FAM) azide, Copper (II)-TBTA complex and ascorbic acid were obtained from Lumiprobe Corporation. Sodium azide and Ciprofloxacin Hydrochloride were purchased from Sangon Biotechnology Inc. (Shanghai, China). *Staphylococcus aureus* (*S. aureus*, ATCC 25923) and *Escherichia coli* (*E. coli*, ATCC 25922) were purchased from Chuanxiang Biotechnology, Ltd (Shanghai, China). *Bacillus subtilis* (*B. subtilis*, ATCC 6633) were purchased from American Type Culture Collection (ATCC).

Measurements and characterizations

A field emission scanning electron microscope (FESEM, S4800, Hitachi) was applied to determine the morphology of the as-prepared samples. TEM was performed on a JEOL 1011 transmission electron microscope at an accelerating voltage of 200 kV. N_2 adsorption-desorption isotherms were recorded on a Micromeritics ASAP 2020M automated sorption analyzer. UV-Vis absorption spectra were recorded using a Varian

Cary300 spectrophotometer equipped with a 1 cm path length quartz cell. ^{1}H NMR spectra were recorded on Bruker AV400 NMR spectrometer. Fourier transform infrared (FT-IR) spectra were recorded on a BioRad Win-IR instrument using KBr method.

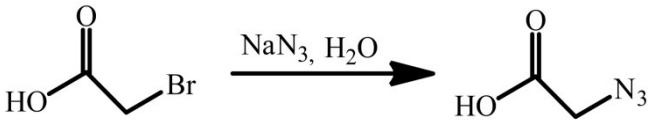
Synthesis of Fe_3O_4@mSiO_2 nanoparticles (FMSN)

The monodisperse Fe_3O_4@mSiO_2 were prepared according to our previous report.[20]

Synthesis of FITC-labeled nanoparticles

1 mg FITC was reacted with 22 μL of APTES in 1 mL ethanol for 2 h in the dark. Then, 20 μL was added into FMSN solution (2 mg/ml, 10 mL). Finally, ammonia (50 μL) was added and the mixture was stirred at room temperature for 24 h, followed by centrifugation and washing with water.

The synthesis of 2-azidoacetic acid



Sodium azide (6.95 g, 50 mmol) was dissolved in 30 mL of distilled water and cooled to 0°C. Bromoacetic acid (7.15 g, 100 mmol) was then added over 10 mins and the reaction was allowed to slowly warm to room temperature overnight. The reaction was acidified to pH = 1 and extracted with 5 × 10 mL diethyl ether. The organic layers were combined, dried over $MgSO_4$, and concentrated to afford 2-azidoacetic acid as a colorless oil (70% yield). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.87 (s, 1H), 3.97 (s, 2H).

Chemical modification of the FMSN surface

To get amino-modified nanopaticles, FMSN (1 g) was refluxed for 24 h in 100 mL of anhydrous toluene with 1 mL of 3-aminopropyltrimethoxysilane (APTES). The FMSN-NH_2 (50 mg) was reacted with succinic anhydride (1.00 g) in dry N,N-dimetylformamide solution (20 mL) under N_2 gas for 8 h with continuous stirring. By doing so, carboxyl groups were formed onto the FMSN surface for conjugation of TTDDA. After a thorough water wash, the carboxylated nanoparticles (FMSN-COOH) were dry and activated using EDC (10 mg/mL, 15 mL) in DMSO. Then 1.5 mLTTDDA was added at room temperature with continuous stirring for 24 h. The FMSN-TTDDA were then centrifuged and washed thoroughly with water. 2-azidoacetic acid and EDC (molar ratio of 2-azidoacetic acid: EDC = 1:2) were dissolved in anhydrous dimethyl sulfoxide (DMSO, 5 mL) under continuous stirring to activate the COOH group of 2-azidoacetic acid. Then the activated 2-azidoacetic acid was added dropwise into the FMSN-TTDDA solution. The resultant mixture was stirred at room temperature for about 24 h. FMSN-TTDDA-N_3 was obtained by centrifugation.

Preparation of Ciprofloxacin Hydrochloride (Cip) loaded FMSN-TTDDA-N_3 (FMSN@Cip-TTDDA-N_3)

4 mg of FMSN-TTDDA-N_3 were mixed with 10 mM of Cip solution (200 µL) in PBS buffer (pH 5.6). After stirred for 24 h, the Cip-loaded particles were collected by centrifugation. To evaluate the Cip loading capacity, the supernatant solution was collected and the residual Cip content was measured by UV-Vis measurements at the wavelength of 277 nm.

Drug releasing experiments

For Cip releasing from nanoparticles, the Cip-loaded samples were incubated in PBS for different periods of time. Cip released from nanoparticles were collected by centrifugation at 12000 rpm for 10 min. The amounts of released Cip in the supernatant solution were measured by UV–Vis spectrophotometry.

Bacteria Culture

Before each microbiological experiment, all samples and glassware were sterilized by autoclaving at 120°C for 30 min. The three bacteria strains were grown in LB medium at 37°C. The resultant bacterial cells were isolated by centrifugation (3500 rpm). The bacterial cells were rinsed with PBS buffer, and designed bacterial concentration was adjusted by measuring the optical density at 600 nm (OD_{600nm}).

Cellular imaging to determine the generated alkynyl groups

For labeling experiments, exponentially growing bacteria (OD_{600}~0.3) were mixed with 250 μM clickable ethynyl-L-alanine (ELA) or ethynyl-D-alanine (EDA) and incubated for 40 min. Bacteria were collect by centrifugation and washed with PBS for three times. Bacteria were resuspended in a reaction mixture of half the volume of the original culture. This mixture contained 128 μM Copper (II)-TBTA complex, 1.2 mM freshly prepared sodium ascorbate, and 20 μM of FAM azide in PBS. CuAAC reactions were performed at RT for 45 min. The images were captured using an Olympus BX-51 optical equipped with a CCD camera.

Cellular imaging to show the binding of nanoparticles

The same procedure was followed with the addition of FITC labelled FMSN-TTDDA-N_3 instead of FAM azide.

Targeting bacteria over mammalian cells

HeLa cells were seeded at 5×10^4 cells per well in 24-well plates for 24 h. *B. subtilis* (OD_{600}~0.3) and 1 mM clickable ELA or EDA were added into the plates. HeLa cells and bacteria were incubated in DMEM medium containing 10% LB medium for 1 h at 37°C. Then, the bacteria were harvested by centrifugation and washed with PBS. The cells were rinsed twice with PBS and mixed with the suspended bacteria. Finally, a reaction mixture containing 128 μM Copper (II)-TBTA complex, 1.2 mM freshly prepared sodium ascorbate, and FITC labelled FMSN-TTDDA-N_3 (50 μg/mL) were added into the coculture system of HeLa cells and bacteria. The mixture was incubated for 1 h at 37°C. The images were captured using an Olympus BX-51 optical equipped with a CCD camera.

Selective binding and separation of bacteria from mammalian cells

HeLa cells were seeded onto 20 mm-diameter polystyrene tissue culture plates at a density of 1.5 x 10^6 cells per plate. *B. subtilis* (OD_{600}~0.3) and 1 mM clickable EDA were added into the plates. HeLa cells and bacteria were incubated in DMEM medium containing 10% LB medium for 1 h at 37°C. Then, the bacteria was harvested by centrifugation and washed with PBS. The cells were rinsed twice with PBS and mixed with the suspended bacteria. Finally, a reaction mixture containing 128 μM Copper (II)-TBTA complex, 1.2 mM freshly prepared sodium ascorbate, and FITC labelled FMSN-TTDDA-N_3 (50 μg/mL) were added into the coculture system of HeLa cells

and bacteria. The mixture was incubated for 1 h at 37°C. The images of HeLa cells and *B. subtilis* in a culture dish were taken after incubation with FITC labelled FMSN-TTDDA-N_3 in the presence of magnetic field using an Olympus BX-51 optical equipped with a CCD camera.

Antibacterial Experiments

For the antibacterial experiments, exponentially growing bacteria or EDA-labelled bacteria (OD_{600}~0.3) were added into 96-well assay plates (100 μL total volume/well). Then Cip or FMSN@Cip-TTDDA-N_3 at the indicated concentrations were added, giving the final volume of 200 μL. As for the EDA-labelled bacteria groups the CuAAC reaction mixture was also added. At designed time intervals, absorbance values were determined with Bio-Rad model-680 microplate reader at 570 nm. Six replicates were done for each treatment group.

The viability were conducted by observing the number of colony-forming units on an LB agar plate. EDA-labelled bacteria (OD_{600}~0.3) were mixed with FMSN@Cip-TTDDA-N_3 and CuAAC reaction mixture was also added. After 24 h incubation, the mixture were placed on solid medium by spread plate method and cultured for another 24 h before observing the number of the bacteria colonies. Control experiments were performed in parallel without nanoparticles, FMSN-TTDDA-N_3, Copper (II)-TBTA complex and Cip alone.

Selective binding and killing bacteria over mammalian cells

For the co-culture test (HeLa cells-*S. aureus*), the concentration of cancer cells were fixed at a density of 10^5 cells per well in 24-well assay plates and infected with *S.*

aureus. EDA were added into the plates. HeLa cells and bacteria were incubated in DMEM medium containing 10% LB medium for 1 h at 37°C. Then, the bacteria was harvested by centrifugation and washed with PBS. The cells were rinsed twice with PBS and mixed with the suspended bacteria. FMSN@Cip-TTDDA-N_3 and CuAAC reaction mixture were then added. Co-cultures were incubated at 37°C and 5% CO_2. Images of HeLa cells in the presence of *S. aureus* were recorded at 24 h using an Olympus BX-51 optical system microscope. For the cellular viability analysis, the supernatant was removed. The remaining cells were rinsed twice with PBS, then digested by trypsin and resuspended in fresh complete medium. HeLa cells cellular viability were determined by trypan blue exclusion in a haemocytometer chamber.

Statistical analysis

All data were expressed in this article as mean result ± standard deviation (SD). All Figures shown in this article were obtained from three independent experiments with similar results. The statistical analysis was performed by using Origin 8.0 software.

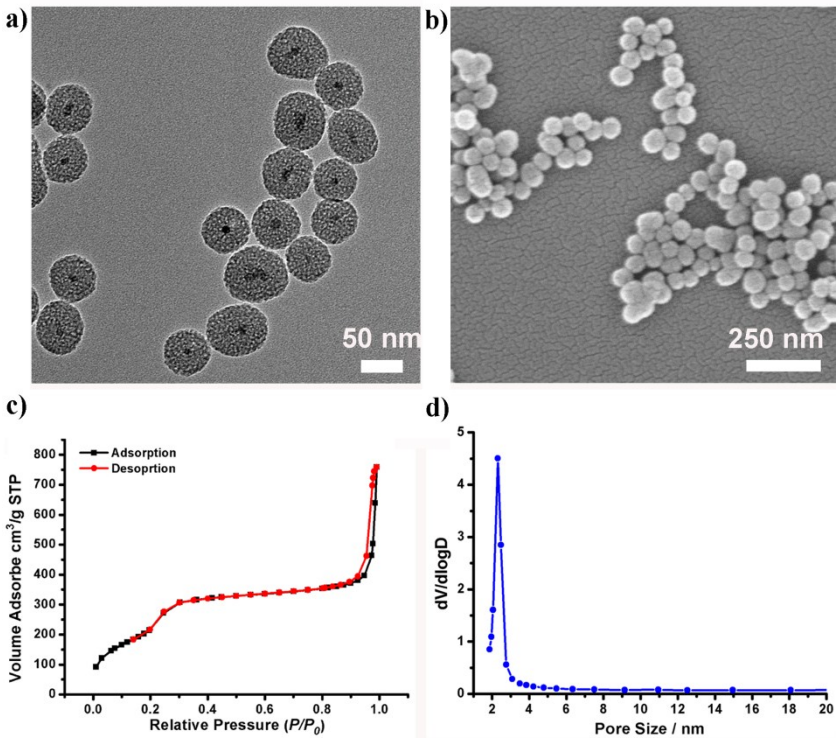


Fig. S1 TEM (a) and SEM images (b) of core-shell FMSNS. N_2 adsorption/desorption isotherms (c) and pore size distribution from adsorption branch (d). N_2 adsorption-desorption isotherms (Figure S1) showed a typical type-IV curve with average pore diameter of 2.3 nm.

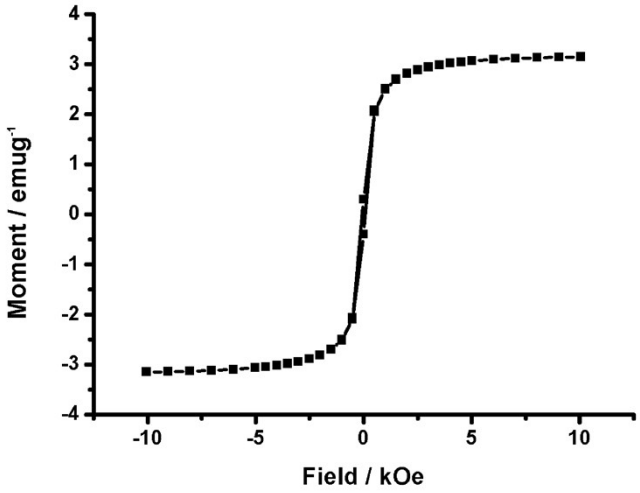


Fig. S2 Field-dependent magnetization at 300 K. Field-dependent magnetism of FMSN particles at 300 K showed no hysteresis, demonstrating that they were superparamagnetic.

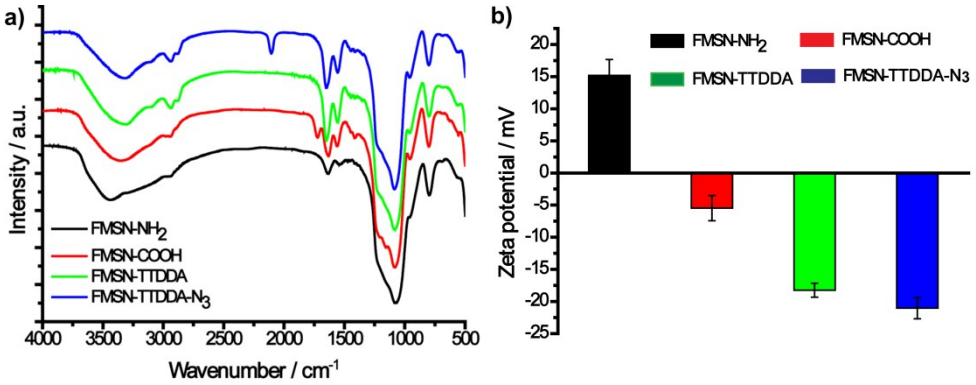


Fig. S3 a) FTIR spectra of FMSN-NH_2 (black), FMSN-COOH (red) TTDDA conjugated FMSN (green) and 2-azidoacetic acid conjugated FMSN (blue). The successful grafting of azide groups onto the FMSN-TTDDA were validated by FTIR spectroscopy. After surface functionalization with 2-azidoacetic acid, the appearance of new peak at 2100 cm^{-1}, which were a typical vibration of azide, proved the successful conjugation of 2-azidoacetic acid with FMSN-TTDDA. b) The zeta potential of different nanoparticles. The amino-functionalized nanoparticles was positively charged (+15.18 ± 2.47 mV) and the zeta potential became negatively charged after the conjugation of –COOH and short-chain PEG (TTDDA).

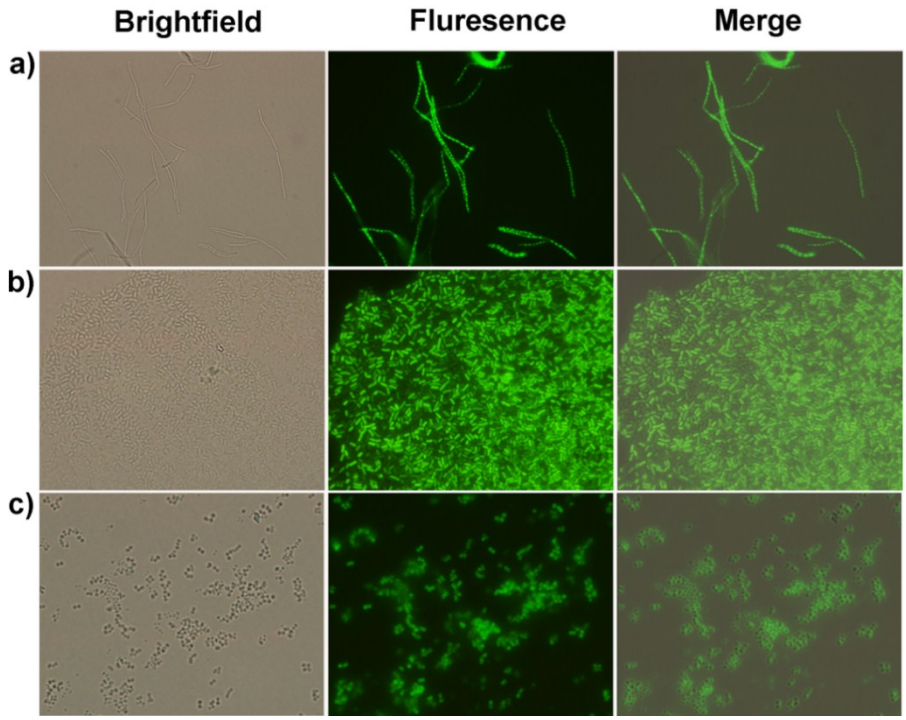


Fig. S4 Incubation of bacteria in D-alanine analogues followed by reaction with click chemistry nanoprobes results in cell surface fluorescence: (a) *B. subtilis*; (b) *E. coli*; (c) *S. aureus*.

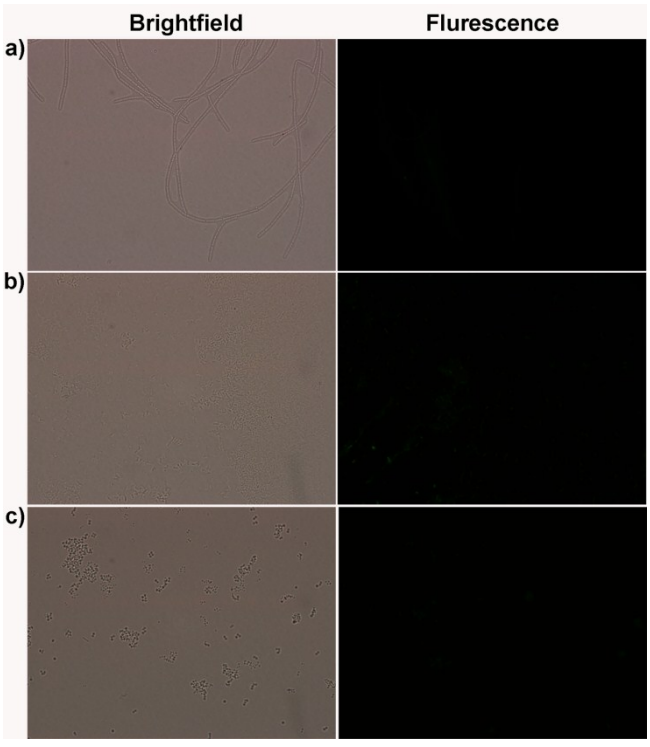


Fig. S5 Incubation of bacteria in L-alanine analogues followed by reaction with click chemistry nanoprobes: (a) *B. subtilis*; (b) *E. coli*; (c) *S. aureus*.

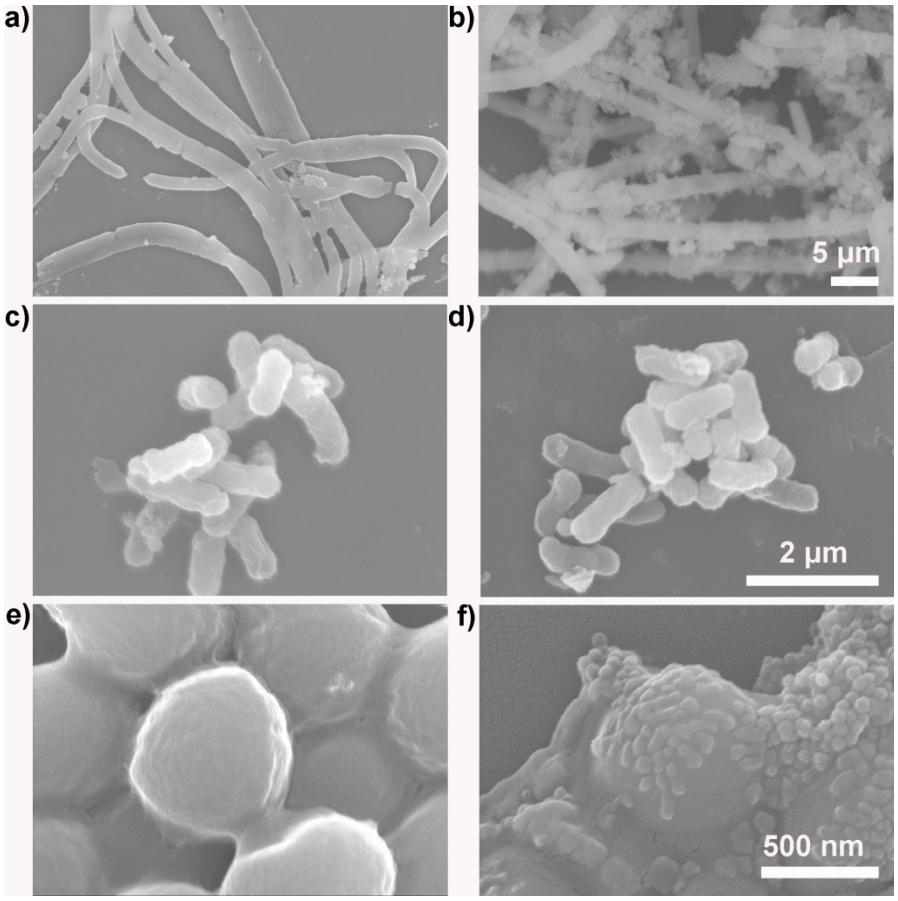


Fig. S6 SEM images of FMSN-TTDDA-N_3 nanoparticles binding to D or ELA-treated bacteria by bioorthogonal click reaction. Nanoparticles binding to ELA-treated *B. subtilis* (a); binding to EDA-treated *B. subtilis* (b); binding to ELA-treated *E. coli* (c); binding to EDA-treated *E. coli* (d); binding to ELA-treated *S. aureus* (e); binding to EDA-treated *S. aureus* (f).

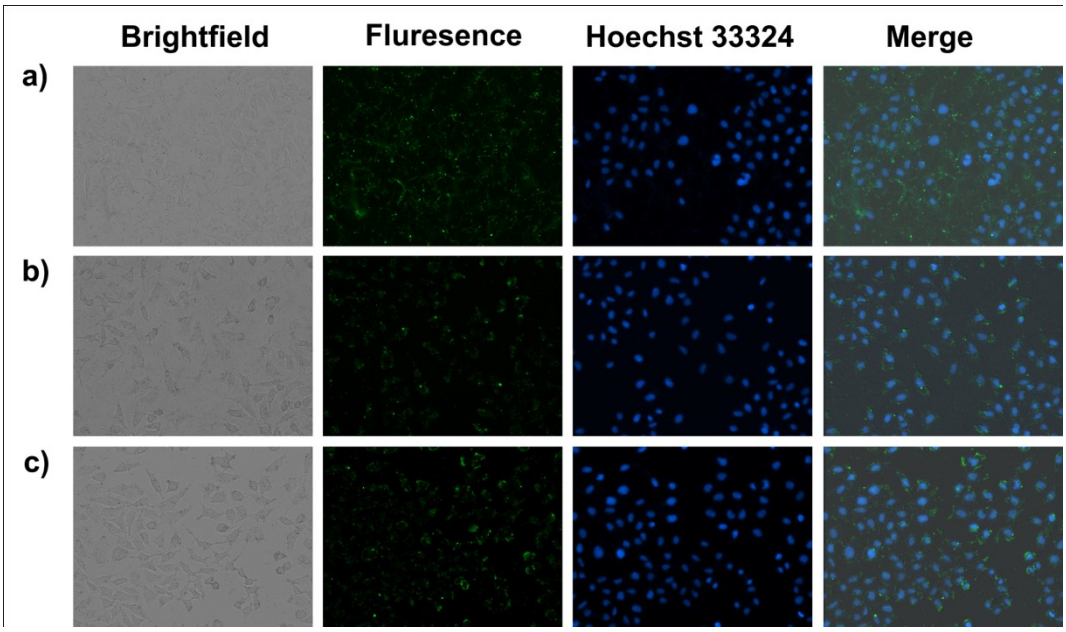


Fig. S7 Fluorescence microscopy images of EDA-treated co-culture system was captured by FMSN-TTDDA-N_3 nanoparticles (a); ELA-treated co-culture system was captured by FMSN-TTDDA-N_3 nanoparticles (b); HeLa cells incubated with FMSN-TTDDA-N_3 nanoparticles (c).

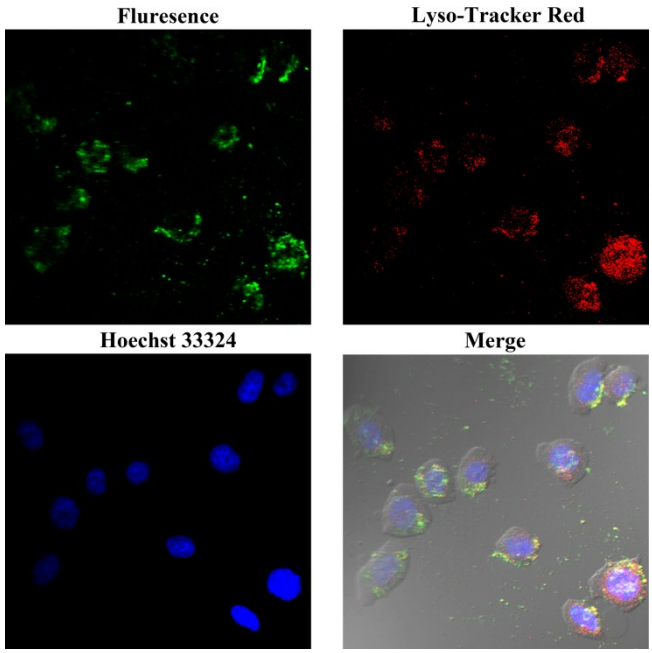


Fig S8 Localization of FMSN-TTDDA-N_3 nanoparticles at endosomes/lysosomes in HeLa cells observed by confocal laser scanning microscopy images

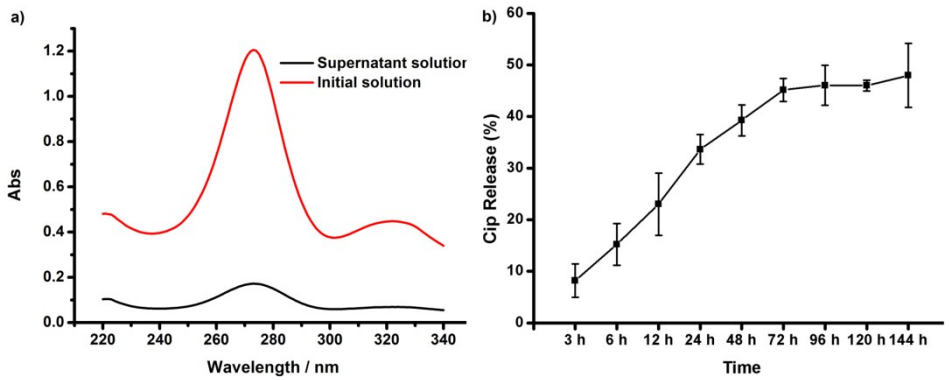


Fig. S9 a) UV-Vis absorption spectra of initial of Cip solution and supernatant Cip solution after loading into FMSN; b) Release profiles of Cip from Cip-loaded FMSN-TTDDA-N3 in PBS buffer.

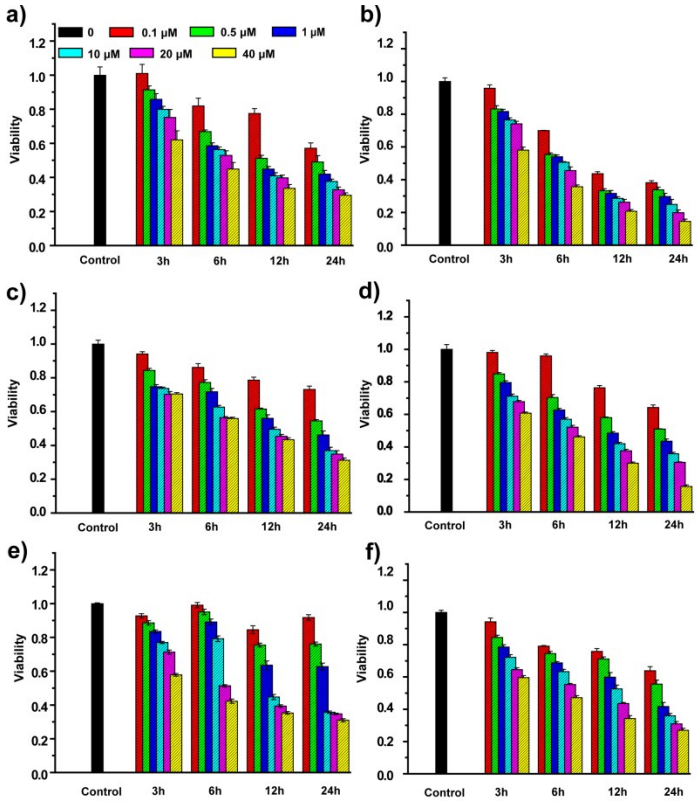


Fig. S10 Bacteria viability of EDA-treated *B. subtilis* (a and b), *S. aureus* (c and d) and *E. coli* (e and f) incubated with Cip alone (a, c and e) and Cip-loaded FMSN-TTDDA-N_3 nanocarriers (b, d and f) at different concentrations.

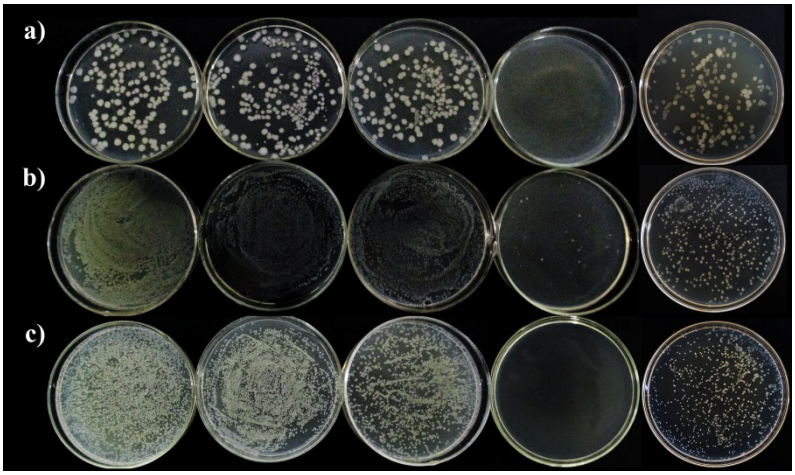


Fig. S11 The plate samples showed colonies of EDA-treated *B. subtilis* (a), *E. coli* (b) and *S. aureus* (c) incubated with PBS, FMSN-TTDDA-N_3 nanoparticles, Cu-TBTA, Cip-loaded FMSN-TTDDA-N_3 nanocarriers and Cip alone (from left to right).

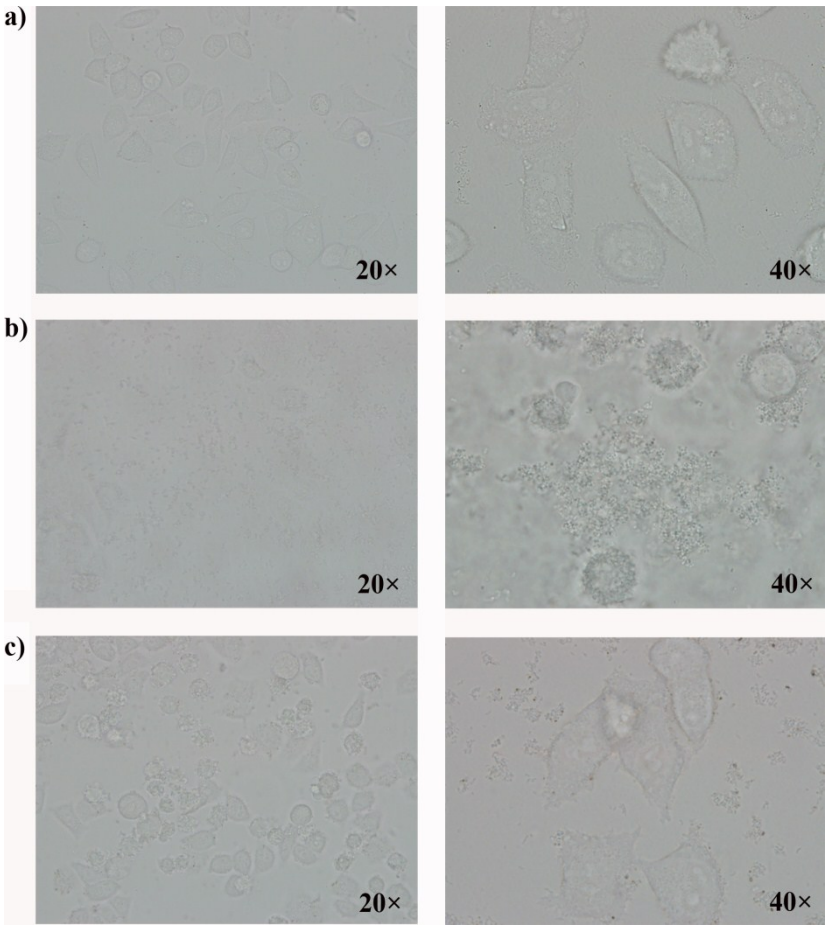


Fig. S12. A co-culture experiment to assess the selective antibacterial effects of the FMSN@Cip-TTDDA-N_3 over mammalian cells. HeLa cells alone (a); EDA-treated

mixture of *S. aureus* and HeLa cells without FMSN@Cip-TTDDA-N_3 (b); EDA-treated mixture of *S. aureus* and HeLa cells with FMSN@Cip-TTDDA-N_3 and CuAAC reaction (c).

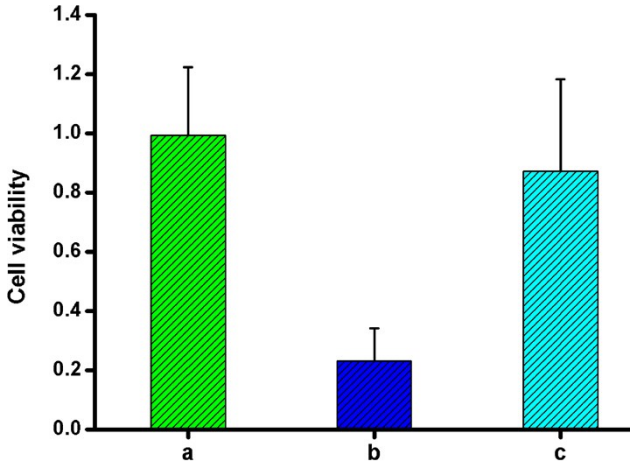


Fig. S13 Cell viability assay of Hela cells in co-culture system. HeLa cells alone (a); EDA-treated mixture of *S. aureus* and HeLa cells without FMSN@Cip-TTDDA-N_3 (b); EDA-treated mixture of *S. aureus* and HeLa cells with FMSN@Cip-TTDDA-N_3 and CuAAC reaction (c).