Multifunctional Upconverting Nanoparticles for Near-Infrared Triggered and Synergistic Antibacterial Resistance Threat
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Experimental section

Materials: MnCl\textsubscript{2}·4H\textsubscript{2}O (99%), Y(NO\textsubscript{3})\textsubscript{3}·6H\textsubscript{2}O (99.9%), Yb(NO\textsubscript{3})\textsubscript{3}·xH\textsubscript{2}O (x=5, 99.99%), Er(NO\textsubscript{3})\textsubscript{3}·xH\textsubscript{2}O (x=5, 99.9%), and oleic acid (technical grade, 90%) were all supplied by Alfa Aesar Reagent Company and used without further purification. Copper sulfate pentahydrate (CuSO\textsubscript{4}·5H\textsubscript{2}O), succinic acid and sodium hydroxide (NaOH) were purchased from Sigma-Aldrich. Hydrazine monohydrate (N\textsubscript{2}H\textsubscript{4}·H\textsubscript{2}O), ammonium sulfide solution (NH\textsubscript{4}S, 20 wt%), ethanol, cyclohexane, chloroform, and NaF were purchased from Aladdin Reagent (Shanghai, China). Water throughout all experiments was obtained by using a Milli-Q water system.

Synthesis of the upconversion nanoparticles (UCNPs): In a typical synthesis route, x ml of 0.5 M MnCl\textsubscript{2}, (1.6-x) ml of 0.5 M Y(NO\textsubscript{3})\textsubscript{3}, 0.9 ml of 0.2 M Yb(NO\textsubscript{3})\textsubscript{3} and 0.1 ml of 0.2 M Er(NO\textsubscript{3})\textsubscript{3} were added to a mixture of NaOH (0.3 g), deionized water (1.5 ml), oleic acid (5 ml) and ethanol (10 ml) under thorough stirring. Then, 2 ml of deionized water contained 4 mmol of NaF was dropwise added to the mixture. After vigorous stirring at room temperature for 15 min, the colloidal solution were transferred into a 25 ml Teflon-lined autoclave, sealed and heated at 200 °C for 8h. The systems were then allowed to naturally cool to room temperature. The final products were collected by means of centrifugation, washed with ethanol and deionized water for several times to remove any possible remnants, and then dried in vacuum at 70 °C to obtain the dried UCNPs powder.

Synthesis of NaYF\textsubscript{4}:Er/Yb/Mn@SiO\textsubscript{2}(MB): 50mg upconversion nanoparticles were added to the 1.8ml tritonX-100, 7.5 ml cyclohexane, 1.8 ml hexanol and 340 μl H\textsubscript{2}O mixture under magnetic stir. Under gentle stirring, concentrated methylene blue (MB; 20ml) aqueous solution was added and stirred for one additional hour. Then, ammonia (200 μl, 25 wt %) and TEOS (100 μl) were added consecutively. The reaction was stirred overnight at room temperature. To graft amino-groups, 50 μl APTES was added into the system and stirred for another 5 h. The tags were separated from the reaction mixture by centrifugation for 10 min at 10000 rpm, washed twice with EtOH, and then redispersed in EtOH (1 ml).
Preparation of UCNPs/MB/CuS with electrostatic adsorption technique: Firstly, negatively charged citrate-stablized CuS nanoparticles were synthesized using previously reported procedures. Na$_2$S·9H$_2$O solution (2 ml, 0.05 M) was added into 100 ml of the aqueous solution of CuCl$_2$·2H$_2$O (17 mg, 0.1 mmol) and sodium citrate (0.02 g, 0.068 mmol) with stirring at the room temperature. After 5 min, the dark-brown mixture was transferred to the water bath at the temperature of 80 °C and stirred for 10 min until the color of solution turn into dark-green. Then, the mixture was immediately placed in the ice-cold water. The as-prepared CuS solution (60 ml) was mixed with UCNP@SiO$_2$-NH$_2$ (10 ml) with stirring for 40 min. The products were collected by centrifugation and dispersed in the 20 ml deionized water. Then succinic acid was added, and the mixture was stirred for another 12 h at the room temperature.

Chitosan (CS) modified UCNPs/MB/CuS: UCNPs/MB/CuS-CS was prepared by the amidation of UCNPs/MB/CuS with CS in the presence of EDC and NHS. In a typical procedure, CS (0.5 g, 2.77 mmol) and UCNPs/MB/CuS (0.1 g, 0.17 mmol) were firstly dispersed in 50 ml of MES buffer (0.1 M, pH adjusted to 5) and sonicated for 1 h to get a homogeneous colloidal suspension. Being protected by argon, EDC (0.652 g, 3.4 mmol) and NHS (0.782 g, 6.8 mmol) were gradually charged into the flask within 20 min. The reaction was conducted at room temperature under bath sonica-tion for 6 h and stirring for another 16 h. The products were collected by centrifugation and dispersed in the 20 ml deionized water.

Detection of singlet oxygen generation: Detection of singlet oxygen (¹O$_2$) was carried out by using 1,3-diphenylisobenzofuran (DPBF) as a chemical ¹O$_2$ probe, which reacts irreversibly with ¹O$_2$ to cause a decrease in the intensity of the DPBF absorption band at about 400 nm. In a typical experiment, DPBF in acetonitrile (20μl, 8mM) was added to a solution of UCNPs/MB/CuS-Cis in acetonitrile (2 mL). The solution was then irradiated with a 980 nm laser for 10 min. The absorption spectra of the mixture after NIR-laser irradiation were obtained. Free MB and UCNPs/MB/CuS-Cis in acetonitrile mixed with DPBF (20 m L) were introduced as control experiments.

Bacteria Culture: S. aureus and E. coli were grown in LB (Luria Bertani) medium at 37°C, and harvested in the midexponential growth phase. Cultures were centrifuged at 6000 rpm for 10 min to pellet cells, and cells were washed three times with isotonic saline solution to remove residual macromolecules and other growth medium constituents. The pellets were then resuspended in
isotonic saline solution. Bacterial cell suspensions were diluted to obtain cell samples containing $10^6$ to $10^7$ CFU/mL.

Bacteria Viability Test: S. aureus and E. coli cells were incubated with different concentrations of UCNPs/MB/CuS-Cis dispersions in isotonic saline solutions at 37°C. Then the samples were irradiated 980nm light intensity of 1 W cm$^{-2}$ for 10 min. The loss of viability of S. aureus and E. coli cells were evaluated by colony counting method. The irradiated solution was collected, diluted 50 times, spread on agar plates, and incubated for 24 h at 37 °C. The control set was prepared by diluting 200 μl of bacteria to 1 ml with DI water and spread on agar plates.

Characterization of hydrophilic MTUNs nanospheres: A field emission scanning electron microscope (FESEM, S4800, Hitachi) equipped with an energy-dispersive X-ray spectrum was applied to determine the morphology and composition of the as-prepared samples. The SEM samples were prepared by depositing a dilute aqueous dispersion of the as-prepared samples onto a silicon wafer. FT-IR analyses were carried out on a Bruker Vertex 70 FT-IR Spectrometer. UV-Vis-NIR spectroscopy was carried out with a Cary V-550 UV-Vis-NIR spectrometer. All the measurements were performed at room temperature.
**Figure S1**

SEM micrographs of UCNPs/MB (a) and UCNPs/MB/CuS-Cis (b) particles.

**Figure S2**

EDX spectrum of UCNPs/MB/CuS-Cis nanoparticles.

**Figure S3**

Particle size distribution analysis of UCNPs/MB (a) and UCNPs/MB/CuS-Cis (b) particles.
Figure S4

(a) Zeta potential measured at each step of the coating process in deionized water. (b) FTIR spectra of the samples UCNPs/MB-NH$_2$, UCNPs/MB/CuS and UCNPs/MB/CuS-Cis.

Figure S5

Singlet-oxygen generation under NIR-laser irradiation ($\lambda = 980$ nm). Spectra of the change in DPBF absorption around 400 nm upon NIR-laser irradiation (power= 6.0 W) in samples of DPBF, UCNPs/MB and UCNPs/MB/CuS-Cis.
Live/Dead bacterial viability assays of S. aureus treated with UCNPs/MB/CuS-Cis without (a) and with (b) 980nm irradiated for 20 min, and E.coli treated with UCNPs/MB/CuS-Cis without (c) and with (d) 980nm irradiated for 20 min. Scalebar is 20μm.