Electronic Supplementary Material (ESI) for ChemComm.

Lanthanide-doped core-shell nanoparticles as a multimodality platform for imaging and photodynamic therapy†

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Experimental section

Materials: $YCl_3 \cdot 6H_2O$, $YbCl_3 \cdot 6H_2O$, $ErCl_3 \cdot 6H_2O$, $GdCl_3 \cdot 6H_2O$, $NdCl_3 \cdot 6H_2O$, oleic acid, 1-octadecene, (3-aminopropyl)triethoxysilane (APTES), 2-ethoxy-1-ethoxycarbonyl-1, 2-dihydro-quinoline (EEDQ), IGEPAL® CO-520 (M_n = 441 g×mol^{-1}), and 1, 3-diphenylisobenzofuran (DPBF) were all purchased from Sigma-Aldrich without further purification. Rose bengal (RB) and HNO_3 (65%, wt) were obtained from Sinopharm Chemical Reagent Co., China. Tetraethyl orthosilicate (TEOS) was obtained from TCI, China. Arsenazo III was purchased from Shaanxi Keyi, China. Omniscan (Gd-DTPA) was obtained from GE Pharm., USA. Tryptone soy broth (TSB) was purchased from Qingdao Rishui Biotech, China. Two antibiotic-resistant bacterial strains: gram-positive methicillin-resistant *Staphylococcus aureus* (MRSA) and gram-negative extended spectrum β-lactamase (ESBL)-producing *Escherichia coli* were collected from the First Affiliated Hospital of Xi′an Jiaotong

University, Xi′an, China. Nude mouse were supplied by the animal center of Xi'an Jiaotong University. The human bladder cancer cell lines T24, was kindly provided by Dr. Leland W. K. Chung at Cedars Sinai Medical Center, Los Angeles, CA. T24 cell was cultured in Dulbecco's modified Eagles medium (DMEM). All cell lines were supplemented with 10% fetal bovine serum and 100 U/ml 1% penicillin/streptomycin and maintained at 37°C in a 100% humidified atmosphere containing 5% CO_2 at 37°C.

Instruments: Transmission electron microscope (TEM) images were recorded in a Hitachi HT7700 TEM system operating at an acceleration voltage of 100 kV. UV-vis spectra were acquired with a Shimadzu UV-2550 spectrophotometer. Spectra of photoluminescence (PL) were recorded in an Edinburgh FLsp920 spectrophotometer equipped with 808 and 980 nm lasers. Scanning electron microscopy (SEM) images were acquired in an FEI Quanta FEG 250. MRI measurements and scanning of images were obtained using MagnetomSkyra 46283.

Preparation of $NaYF_4$:Yb,Er nanoparticles (NPs): $NaYF_4$:Yb,Er were prepared according to a method reported by our lab with a minor modification. Briefly, $YCl_3 \cdot 6H_2O$ (2 mmol), $YbCl_3 \cdot 6H_2O$ (0.45 mmol), $ErCl_3 \cdot 6H_2O$ (0.05 mmol), oleic acid (27.5 mL), and 1-octadecene (22.5 mL) were added into a 250 mL flask and the mixture was heated at 140°C for 1h under constant stirring and vacuum atmosphere. Then NH_4F (10 mmol) and NaOH (10 mmol) were added. The mixture was heated at 140°C for 1 h, and at 320°C for 1.5 h. The resulting mixture was cooled down to room temperature, and the resulting nanoparticles were collected by centrifugation. After washed with ethanol three times, the nanoparticles were dispersed in cyclohexane.

Preparation of $NaYF_4$:Yb,Er@$NaGdF_4$:Nd core-shell NPs: The core-shell structured $NaYF_4$:Yb,Er@$NaGdF_4$:Nd were synthesized from $NaYF_4$:Yb,Er as described above. In brief, $GdCl_3 \cdot 6H_2O$ (0.64 mmol), $NdCl_3 \cdot 6H_2O$ (0.16 mmol), oleic acid (11 mL) and 1-octadecene (9 mL) were mixed and heated at 140°C for 1 h under stirring. After that, NaF (3.2 mmol) and $NaYF_4$:Yb,Er (0.8 mmol) in cyclohexane were added. The mixture was kept at 120°C for 20 min to evaporate the cyclohexane and heated to 300°C for another 1 h. After cooling down to room temperature, the

desired core-shell NPs were collected by centrifugation, washed with ethanol three times and stored in cyclohexane. The elemental composition of LNPs was determined by EDS.

Preparation of LNPs@SiO_2-RB: Firstly, rose bengal (RB) was conjugated with APTES through an amide linkage to form a RB-APTES precursor as reported.[1] RB (0.05 mol), and EEDQ (0.1 mol) were dissolved in 1 mL methanol, and the mixture was stirred in the dark for 30 min to activate the carboxylic acid of RB. 10 μL of APTES was added into the activated RB, and the mixture was stirred for 24 h at room temperature. The resulting solution was used directly without further purification.

Secondly, the silica coating for LNPs@SiO_2-RB was performed by a Stöber method.[2] To a solution of 5.0 mg LNPs dispersed in 10 mL cyclohexane, 500 μL of IGEPAL® CO-520 was added, and the mixture was ultrasonicated for 5 min prior to the addition of 100 μL of ammonia (25%). Then, TEOS (50 μL) and APTES-RB precursor (50 μL) were added dropwise, and the reaction mixture was shaken for 36 h in the dark at room temperature. The silica/RB-coated LNPs were isolated by centrifugation, washed with ethanol for three times, and stored in ethanol.

Quantitative analysis of Gd^{3+} contents in LNPs: The amount of Gd^{3+} doped in LNPs was determined by a spectrophotometric method using Arsenazo III as a chromophoric chelating agent.[3] LNPs@SiO_2-RB (2 mg) in a tetrafluoroethylene tube was digested by adding a mixture of 1 mL HNO_3 (69%) and 0.2 mL H_2O_2, and incubated at 60°C for 8 h. The digested solution was diluted with HNO_3 (2%) to provide an approximately 100–300 ppb level of Gd^{3+}. The sample solution was incubated with 0.05 mM Arsenazo III for 20 min at pH 2.8, and its absorption at 658 nm was measured. The amount of Gd^{3+} was determined by comparison of its absorption to a standard curve prepared by standard Gd^{3+} solutions.

Quantitative analysis of RB contents in LNPs@SiO_2-RB: The amount of RB bound to the nanoparticle was determined by Beer's Law using a standard curve method.

Spectroscopic measurement of sub-tissue luminescence: The sub-tissue penetration depth of the luminescence emitted by LNPs@SiO_2-RB was investigated

using a FLsp920 spectrophotometer. Similar to a reported method, the luminescence measurement was performed using an experimental setup in which a pork tissue of variable thickness was placed between the LNP solution and the optical device for light detection.[4] Two separate NIR lasers (808, 980 nm) were used for dual excitation, and the intensity of DCL and UCL was measured. The relative intensity of luminescence was calculated as I/I_0, where I refers to the luminescence intensity measured at a specific tissue thickness (depth > 0 mm), while I_0 refers to the luminescence intensity measured without a phantom tissue (depth = 0 mm).

Cytotoxicity *in vitro*: An MTT-based cell proliferation assay was performed to assess the cytotoxicity of LNPs@SiO_2-RB *in vitro*. Human T24 cell line was suspended in a Dulbecco′s Modified Eagle Medium (DMEM) medium, seeded at a density of 1×10^4 in a 96-well culture plate and cultured at 37°C under a humidified atmosphere supplemented with 5% CO_2 for 24 h. The culture medium was replaced with a solution of 100 μL LNPs@SiO_2-RB (0, 100, 200, 300, 400, 500μg/mL) dispersed in a fresh culture medium. The LNPs-treated cells continued incubation at 37°C for 24 h and 48 h in the dark, respectively. Then the cells were treated with 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) in a culture medium (5 mg/mL) and incubated at 37°C for 4 h. After removal of the supernatant, 150 μL of DMSO was added into each wells and the plate was shaken for 10 min. Finally, absorbance at 490 nm of each wells was recorded in a microplate reader (BioTek Epoch). The cell viability is calculated as the following formula:

$$\text{Cell Viability (\%)} = \frac{\text{absorbance value of experiment group}}{\text{absorbance value of control group}} \times 100\,\%$$

T_1*-weighted MR imaging *in vitro* and *in vivo: The *in vitro* characterization of the T_1 relaxivity of LNPs@SiO_2-RB was performed according to a previously reported method.[3] Briefly, LNPs@SiO_2-RB dispersed in agar (2%) was added into an array of Eppendorf tubes (2 mL) with variation concentrations of Gd^{3+} (0-0.5 mM). Scanning of T_1-enhanced MR images was conducted using a T_1-weighted spin-echo pulse sequence under a set of standard parameters that include: i) repetition time (TR) = 500, 1000, 1500, and 2000 ms; ii) echo time (TE) = 15.3 ms; iii) number of

excitations (NEX) = 8. The *in vivo* MR images of LNPs@SiO_2-RB were performed in nude mice.[3] All animal experiments were approved by the Institutional Animal Care and Use Committee of Xi'an Jiaotong University and were performed according to the institution's guidelines for the use of laboratory animals. A nude mouse was anesthetized with 10% chloral hydrate (0.6 mL per 150 g), and injected intramuscularly with 100 μL of LNPs@SiO_2-RB (150 μg/mL) or the standard MRI agent. Then the mouse was scanned for MR images before and after LNPs injection using T_1-weighted sequence (multi echo spin echo, MESE) under a set of standard parameters (TR = 400 ms, TE = 5 ms, average times = 8, matrix size = 128 × 128, field of view (FOV) = 3.0 cm × 3.0 cm, slice thickness = 1.0 mm).

Histological analysis: The biocompatibility of LNPs@SiO_2-RB was investigated by performing the histopathological analysis of tissues *in vivo*. Nude mice were divided into test and control groups. Mice in test group were intramuscularly injected with 100 μL of LNPs@SiO_2-RB (150 μg/mL) while mice in control group were untreated. After 7 days of feeding, mice were executed and their organs including heart, liver, spleen, lung, and kidney were harvested. Slices of organ sections were prepared and stained with hematoxylin and eosin (H&E), and examined by optical microscopy.

Efficiency of $^1O_2$ generation: The efficiency of $^1O_2$ production by LNPs@SiO_2-RB was determined in a spectrophotometric assay by using DPBF as a fluorescent probe. DPBF has a specific reactivity for $^1O_2$, forming an irreversible adduct which is non-fluorescent.[5] In this assay, 3 mL of LNPs@SiO_2-RB (100 μg/mL) dissolved in DMSO was placed into a 5 mL cuvette to acquire its UV-vis absorption spectrum. Then, 15 μL of DPBF in DMSO (5 mM) was added and an additional absorption spectrum was acquired. The mixture was then irradiated by a 980 or 808 nm NIR laser at 1 W/cm^2 and its UV-Vis absorption spectra were measured as a function of irradiation time with a 5 min interval. The change in absorption at 400 nm of the irradiated solution was calculated as a measure of the degradation rate of DPBF and it was used to determine the efficiency of $^1O_2$ generation.

Photodynamic antibacterial efficacy *in vitro*: Two antibiotic-resistant bacterial strains: MRSA and ESBL-producing *E. coli* on tryptone soy agar (TSA) were inoculated into 10 mL of a fresh TSB and incubated overnight at 37 °C. The bacterial pellets were collected by centrifugation at 4000 rpm for 10 min and washed three times with PBS (pH 7.4). The bacterial cells were diluted to a density of 1×10^7 colony forming units (CFU)/mL before use. Bacterial suspension (0.3 mL) was mixed with 0.3 mL of a LNPs solution at varying concentrations and the mixture was incubated at 37°C for 2 h in the dark. The mixture solution was exposed to a 980 nm NIR laser with the light dose of 1 W/cm^2 for 20 min. The treated cells were collected by centrifugation at 4000 rpm for 10 min and resuspended in sterilized PBS. Bacterial suspension without the LNPs@SiO_2-RB treatment was used as a control. The bacterial cells were then serially diluted 10-fold with sterilized PBS and 20 μL from each dilution was spread on the TSA in triplicate. The bacterial colonies were grown at 37°C for 24 h and the number of CFUs was counted. Viability is defined as the ratio of N_T/N_0, where N_T refers to the number of CFU after photodynamic treatment, and N_0 refers to the number of CFU without any treatment.

Scanning Electron Microscopy (SEM): The shapes and surface morphologies of bacterial cells were examined by SEM. After irradiation, the bacterial pellets was collected by centrifugation and washed twice with PBS. Then the pellets were fixed in a glutaraldehyde (1.0%, wt) aqueous solution at 4°C for 1 h. Each sample was washed thrice with PBS, and dehydrated in aqueous ethanol in a graded manner (30, 70, 90, and 100%) and in tertiary-butanol at 4°C for 20 min. Finally, the samples were freeze dried, sputter coated with gold and observed using SEM.

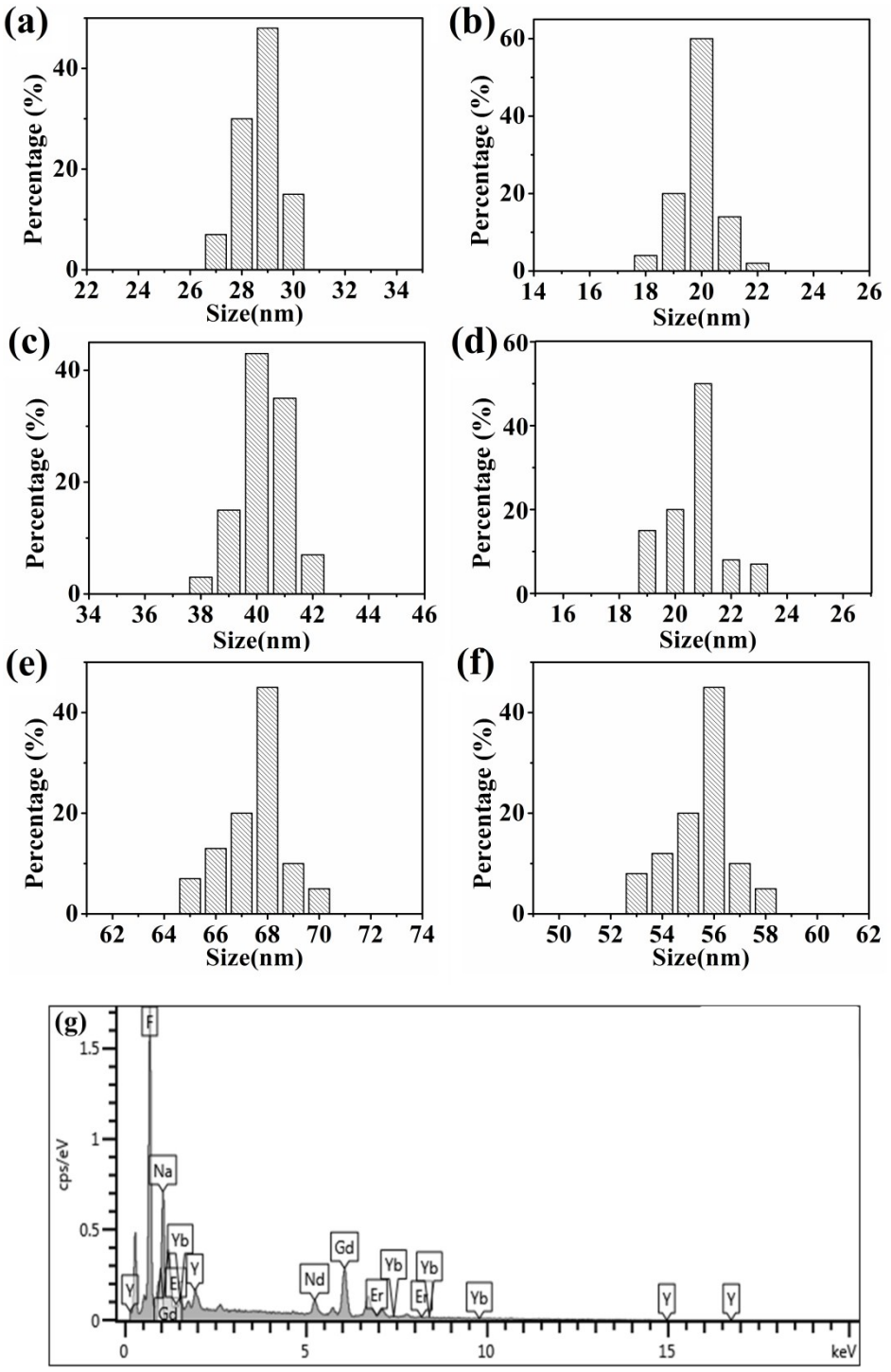

Fig. S1 Size distributions of (a, b) core $NaYF_4$:Yb,Er; (c, d) core-shell $NaYF_4$:Yb,Er@$NaGdF_4$:Nd; (e, f) LNPs@SiO_2-RB. The size in plots of (a, c, and e) refers to the length and the size in the plots for (b, d, f) refers to the width of LNPs. (g) The EDS plot of $NaYF_4$:Yb,Er@$NaGdF_4$:Nd.

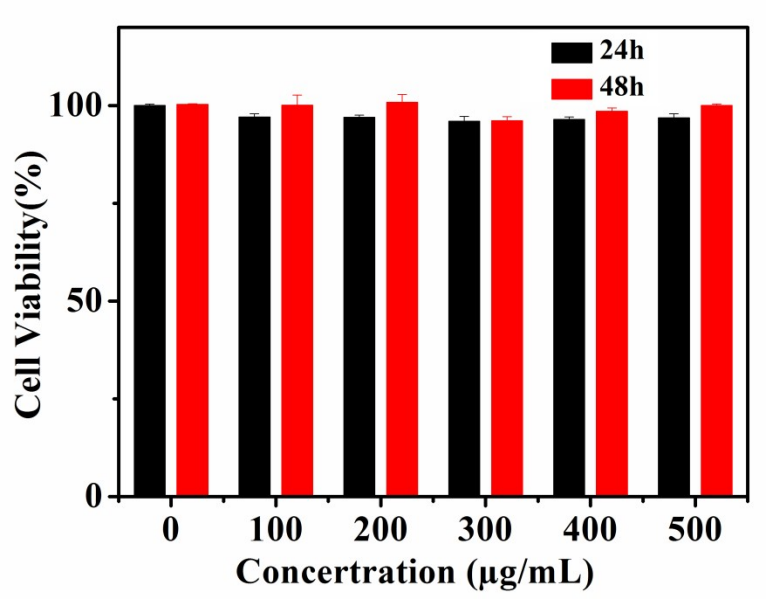

Fig. S2. *In vitro* cell viability of T24 cells (human bladder carcinoma) after treatment with LNPs@SiO_2-RB in the dark for 24 h and 48 h.

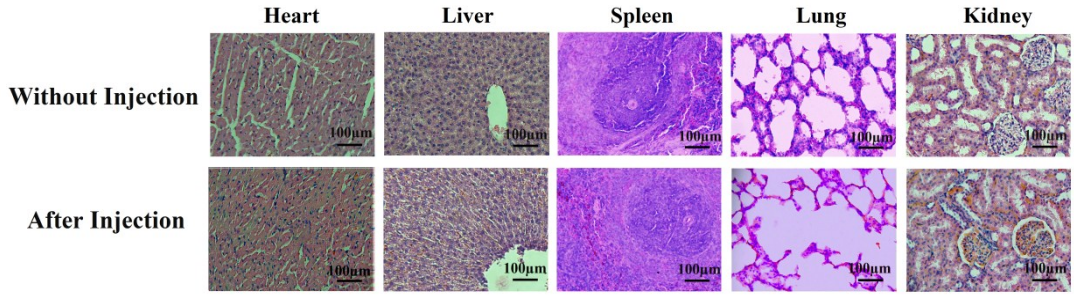

Fig. S3 Histological analysis of the tissues harvested from a mouse after treatment with or without LNPs@SiO_2-RB injection. Each image shows a H&E-stained tissue section of the heart, liver, spleen, lung, and kidney harvested from mouse with or without the nanoparticles injection.

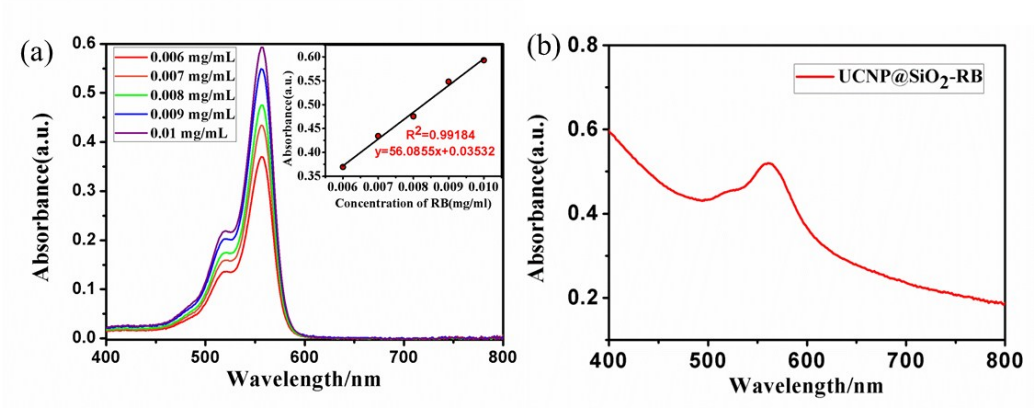

Fig. S4 (a) The RB absorption spectrum and its standard curve in deionized water. (b) the absorption spectrum of LNPs@SiO_2-RB in deionized water.

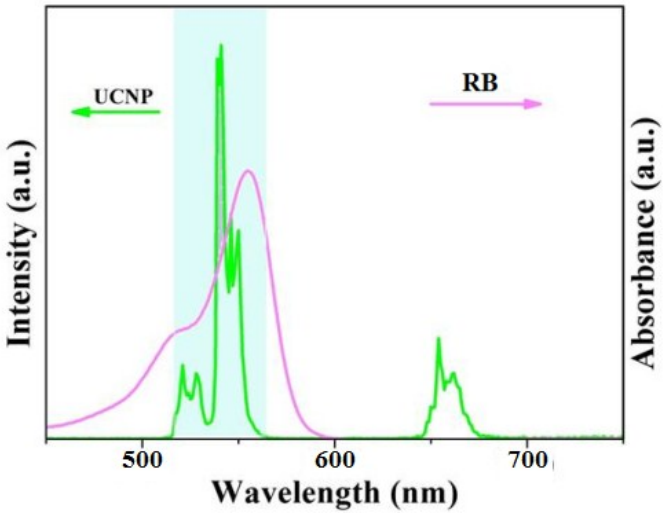

Fig. S5 UCL emission spectrum of the LNPs in cyclohexane (green), and the absorption spectrum of RB molecules (red) in deionized water.

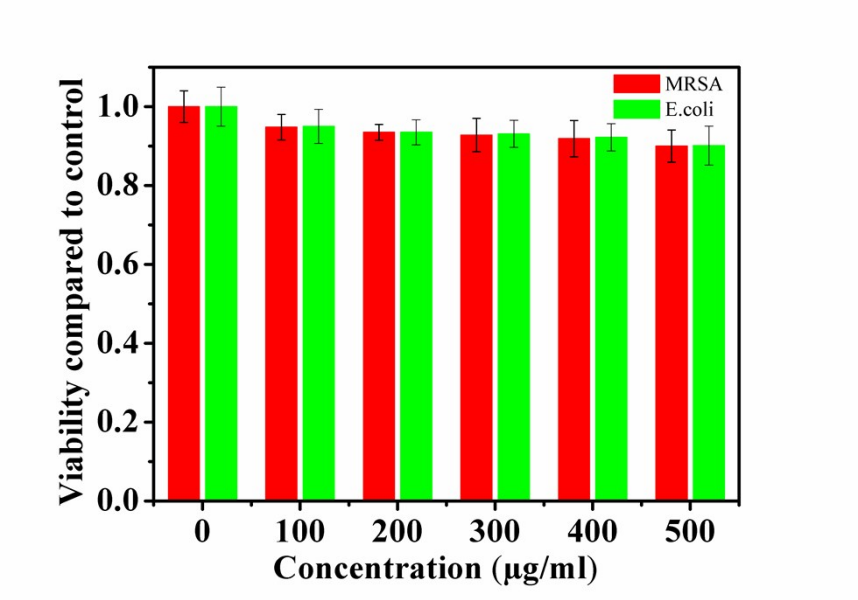

Fig. S6 Cytotoxicity assay of MRSA and ESBL-producing *E. coli* treated with LNPs@SiO_2-RB as a function of concentrations (non irradiation).

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