Supporting Information

Magneto-Fluorescent Bacteria Assay Strategy based on Dual Colour Sulfide Fluorescent Nanoparticles with High Near-IR Conversion Efficiency

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Experimental Section

Reagents

All starting materials, unless mentioned otherwise, were obtained from commercial supplies and used directly. Europium (III) nitrate ($Eu(NO_3)_3$, 99.99 %), Samarium (III) nitrate ($Sm(NO_3)_3$, 99.99 %), Cerium (III) nitrate ($Ce(NO_3)_3$, 99.95 %), Iron chloride ($FeCl_3$), Zinc chloride ($ZnCl_2$, 98 %), Hexadecyltrimethyl ammonium bromide (CTAB, 99 %), 3-Mercaptopropionic Acid (3-MPA, 98 %), 1-Pentanol (98 %), 1-Dodecanethiol (95 %), Tergitol (NP-10), 3-Mercaptopropionic Acid (99 %), Sodium citrate (Na3Cit, 98%), and Sodium acetate (NaOAc, 98%), N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC·HCl, 98 %), N-Hydroxysuccinimide (NHS, 98 %), 4-(Dimethylamino) pyridine (DAMP, 99 %), were purchased from Aladdin Reagent, Co., Ltd. (Shanghai, China). Cyclohexane (99.5 %), Calcium nitrate tetrahydrate ($Ca(NO_3)_2$•$4H_2O$, 99%), Strontium nitrate

tetrahydrate ($Sr(NO_3)_2$•$4H_2O$, 99 %), Manganese acetate tetrahydrate ($Mn(CH_3COO)_2$•$4H_2O$, 99 %), Ammonium sulfate ($(NH_4)_2SO_4$, 99 %), Sodium hydroxide (NaOH, 96 %) and Nitric acid (HNO_3, 70 %) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Teicoplanin (TEC, >900 ug/mg, BR) and Polymyxin B (PMB, >6500 IU/mg, USP) was purchased from Dalian Meilun Biotechnology Co., Ltd. Amino/Carboxyl-derivatized polyethylene glycol (H_2N-PEG-COOH, M.W 2000) was obtained from Tansh-Tec Regent Co.,Ltd. Ubiquicidin (29-41) was purchased from Apeptides Biotechnology Co.,Ltd. 11-Mercaptoundecanoic acid (3-MUA, 98 %) was purchased from Energy Chemical Reagent Co.,Ltd. (Shanghai, China). Ethanol (anhydrous, 99.7 %) and Acetone (99 %) were obtained from Beijing Chemical Regent Co., Ltd. N,N-dimethyl formamide (DMF, 99.5 %) was obtained from Tianjin Kermel Chemical Co., Ltd. CdS/ZnS QDs (maximum emission wavelength at ~420 nm) and CdSe/ZnS QDs (maximum emission wavelength at ~625 nm) were purchased from Soochow Mesolight Co., Ltd. All aqueous solutions were prepared using ultrapure water (Mill-Q, Millipore, 18.2 MΩ resistivity).

Apparatus

TEM images of the nanomaterials were collected by JEOL JEM-2010 transmission electron microscope at 200 kV. Powder X-ray diffraction patterns were recorded by D8 Advance Bruker powder X-ray diffractometer (Cu Kα radiation, λ=1.5406 Å) from 10° to 80° at scanning rate of 4°/min. The near-infrared stimulated fluorescence emission spectrum were acquired on a Hitachi F-4600 spectrofluorometer with an external 980 nm NIR laser (Changchun New Industries Optoelectronics Technology Co.,Ltd.). The UV-Visible spectrum were acquired on Agilent Cary-60 UV-Vis spectrophotometer. The Mass spectroscopy was collected in Agilent 1200LC Series coupled to 6460 triple quadrupole mass spectrometer. The size distribution and Zeta-potential were measured using Malven Nano Zetasizer system by Malvern Instruments. Fluorescence images of bacteria cell were acquired in Nikon confocal microscopy A1. All fluorescence photographs of the nanoparticles solutions and bacteria suspensions were collected by smartphone equipped with a NIR filter to eliminate 980 nm excitation light.

Synthesis and Encapsulation of CaS NPs

2.75 mmol hexadecyltrimethyl ammonium bromide (CTAB) was dissolved in the 1-pentanol/cyclohexane solution (1.5 mL: 20 mL), then mixed with 0.5 mL aqueous solution of $Ca(NO_3)_2$ (0.1 M), 0.1 mL $Eu(NO_3)_3$ (1.5 mM), 0.1 mL $Sm(NO_3)_3$ (1.5 mM), and 0.26 mL $Mn(CH_3COO)_2$ (1.5 mM). The mixture was stirred vigorously for 30 min forming a water-oil emulsion, and then left to stand for 1 h. Into this was mixed a water-oil emulsion containing $(NH_4)_2SO_4$ (0.55 mL, 0.1 M), followed by slow agitation for 3 min. After aging for 10 min, 10 mL acetone was added to the mixture. The resultant particles were isolated by centrifuge, 5550 g for 10 min, and alternately washed with acetone and ethanol. The precipitate was dried in vacuum and annealed at 850 °C for 60 min under CO flow to acquire the CaS:Eu,Sm,Mn nanoparticles. Under a dry nitrogen atmosphere the as-prepared CaS:Eu,Sm,Mn nanoparticles were then modified with 1-dodecanethiol (DT) by dispersing them in 10 mL absolute ethanol containing 20 μL Tergitol NP-10. After sonication for 30 min, the mixture was added into 5 mL absolute ethanol containing 0.5 mL DT at pH 8.0, obtained by drop-wise addition of 0.25 M NaOH solution in ethanol. The solution was stirred at 50 °C for 24 h, then centrifuged and washed with ethanol, and finally dispersed in 2 mL cyclohexane. The DT-modified CaS:Eu,Sm,Mn nanoparticles were then encapsulated by 3-mercaptoundecanoic acid (MUA). For this purpose, the DT-modified CaS nanoparticles in cyclohexane were added into 8 mL absolute ethanol, and sonicated for 10 min. Then 150 mg MUA and 10 μL 3-mercaptopropionic acid (MPA) were dissolved in 5 mL absolute ethanol adjusted to pH 8.5 with a 0.25 M NaOH ethanol solution. After stirring for 30 min the solution was added to the cyclohexane solution containing the DT-modified CaS:Eu,Sm,Mn nanoparticles, and stirred vigorously for 48 h. The DT/MUA encapsulated CaS:Eu,Sm,Mn nanoparticles (CaS NPs) were collected by centrifuge at 5550 g and washed with ethanol. The product was dispersed in ethanol and centrifuged at 1400 g for 4 min to precipitate aggregated CaS NPs, and could be illuminated by ultraviolet lamp for 3 min to get fully activated.

Synthesis of CaS-TEC NPs

The as-prepared CaS NPs were dis-persed in 5 mL DMF and sonicated for 10 min to obtain a transparent solution. Then EDC·HCl (10 mg) was added to the solution and stirred for 15 min. Afterward, NHS (5 mg) was added and stirred at 37 °C for 2 h. The nanoparticles were isolated via centrifugation at 10000 rpm and re-dispersed in DMF (2.5 mL), in which 2.5 mL DMF containing 25 mg NH_2-PEG-COOH was then added, and stirred for 24 h. The resulting CaS-PEG NPs were collected by centrifugation at 10000 rpm and washed with ethanol three times. The CaS-PEG NPs (~5 mg) were re-dispersed in DMF (1 mL), and reacted with EDC·HCl (10 mg) for 30 min, after-wards DMAP (0.2 mg) and TEC (10 mg) was added into solution, vigorously stirring at room temperature for 24 h. The mixture was dialyzed against deionized water (5 kDa) to acquire CaS-TEC NPs.

Synthesis and Encapsulation of SrS NPs

2.75 mmol hexadecyltrimethyl ammonium bromide (CTAB) was dissolved in the 1-pentanol/cyclohexane solution (1.5 mL: 20 mL), then mixed with 0.5 mL aqueous solution of $Sr(NO_3)_2$ (0.1 M), 75 μL $Ce(NO_3)_2$ (0.5 mM), 0.1 mL $Sm(NO_3)_3$ (1.5 mM), and 0.20 mL $Mn(CH_3COO)_2$ (1.5 mM). The mixture was stirred vigorously for 30 min forming a water-oil emulsion, and then left to stand for 1 h. Into this was mixed a water-oil emulsion containing $(NH_4)_2SO_4$ (0.55 mL, 0.1 M), followed by slow agitation for 3 min. After aging for 10 min, 10 mL acetone was added to the mixture. The resultant particles were isolated by centrifuge, 5550 g for 10 min, and alternately washed with acetone and ethanol. The precipitate was dried in vacuum and annealed at 900 °C for 60 min under CO flow to acquire the SrS:Ce,Sm,Mn nanoparticles. Under a dry nitrogen atmosphere the as-prepared SrS:Ce,Sm,Mn nanoparticles were then modified with 1-dodecanethiol (DT) by dispersing them in 10 mL absolute ethanol containing 20 μL Tergitol NP-10. After sonication for 30 min, the mixture was added into 5 mL absolute ethanol containing 0.5 mL DT at pH 8.0, obtained by drop-wise addition of 0.25 M NaOH solution in ethanol. The solution was stirred at 50 °C for 24 h, then centrifuged and washed with ethanol, and finally dispersed in 2 mL

cyclohexane. The DT-modified SrS:Ce,Sm,Mn nanoparticles were then encapsulated by 3-mercaptoundecanoic acid (MUA). For this purpose, the DT-modified SrS nanoparticles in cyclohexane were added into 8 mL absolute ethanol, and sonicated for 10 min. Then 150 mg MUA and 10 μL 3-mercaptopropionic acid (MPA) were dissolved in 5 mL absolute ethanol adjusted to pH 8.5 with a 0.25 M NaOH ethanol solution. After stirring for 30 min the solution was added to the cyclohexane solution containing the DT-modified SrS:Ce,Sm,Mn nanoparticles, and stirred vigorously for 48 h. The DT/MUA encapsulated SrS:Ce,Sm,Mn nanoparticles (SrS NPs) were collected by centrifuge at 5550 g and washed with ethanol. The product was dispersed in ethanol and centrifuged at 1400 g for 4 min to precipitate aggregated SrS NPs, and could be illuminated by ultraviolet lamp for 3 min to get fully activated.

Synthesis of SrS-PMB NPs

The blue fluorescence emissive SrS NPs were prepared using microemulsion method. The as-prepared SrS NPs were dispersed in 5 mL DMF and sonicated for 10 min to obtain a transparent solution. Then EDC·HCl (10 mg) was added to the solution and stirred for 15 min. Afterward, NHS (5 mg) was added and stirred at 37 °C for 2 h. The nanoparticles were isolated via centrifugation at 10000 rpm and re-dispersed in DMF (2.5 mL), in which 2.5 mL DMF containing 25 mg NH_2-PEG-COOH was then added, and stirred for 24 h. The resulting SrS-PEG NPs were collected by centrifugation at 10000 rpm and washed with ethanol three times. The SrS-PEG NPs (~5 mg) were re-dispersed in DMF (1 mL), and reacted with EDC·HCl (10 mg) for 30 min, afterwards DMAP (0.2 mg) and PMB (10 mg) was added into solution, vigorously stirring at room temperature for 24 h. The mixture was dialyzed against deionized water (5 kDa) to acquire SrS-PMB NPs.

Determination of the Targeting Ligands

The loading capacity of the target ligands (TEC or PMB) on the nanoparticles was determined by HPLC-ESI-MS, the supernatant contain-ing unreacted TEC or PMB was diluted after centrifugation

and filtration. For reference, the sulfide nanoparticles and the targeting ligands were physically blended (without EDC and DMAP activator), and the supernatant was processed consistently. The HPLC-ESI-MS analysis was performed using 1200LC Series (Agilent Technologies, USA) coupled to 6460 triple quadrupole mass spectrometer (Agilent Technologies, USA). Liquid chromatography separations were carried out in Agilent Eclipse Plus C18 column (2.1 mm × 100 mm, 3. 5 μm). The mobile phase for TEC assay was composed of 0.1 % ammonium acetate aqueous solution (eluent A, 15 %) and pure methanol (eluent B, 85 %). The mobile phase for PMB determination was composed of 0.2 % formic acid aqueous solution (eluent A, 65 %) and pure acetonitrile (eluent B, 35 %). The flow rate and temperature of the mobile phase were 0.2 mL/min and 30 °C, respectively. The mass spectrometer was performed in positive ion mode using an electrospray ionization (ESI) source. The acquisition was operated in the single ion monitoring (SIM), $[M+H]^+$ ions were employed for quantitative determination. For the quantitative analysis, the calibration curves were prepared in the range of 1~100 μg/mL for TEC, and 1~50 μg/mL for PMB. The loading capacity of TEC or PMB conjugated on the nanoparticles were calculated based the standard curves, respectively.

Synthesis and Modification of $NaYF_4$:Yb,Er,Mn NPs and $NaYF_4$:Yb,Tm NPs

The red emissive $NaYF_4$:Yb,Er,Mn nanoparticles (r-$NaYF_4$ NPs) were prepared according to the literature,[1] and the obtained r-$NaYF_4$ NPs were dispersed in 5 mL chloroform, incubated with 3-mercaptopropionic acid (5 mL). After the solution was stirred for 24 h, 5 mL deionized water was added to it and it was further stirred for 1h. The MUA modified r-$NaYF_4$ NPs were collected by centrifugation. The carboxyl-terminated nanoparticles (~4 mg) were dispersed in 5 mL DMF and sonicated for 10 min to obtain a transparent solution. Then EDC·HCl (10 mg) was added to the solution and stirred for 15 min. Afterward, NHS (5 mg) was added and stirred at 37°C for 2 h. The nanoparticles were isolated via centrifugation at 5500 g and re-dispersed in DMF (2.5 mL), in which 2.5 mL DMF containing 25 mg NH_2-PEG-COOH was then added, and stirred for 24 h. The resulting PEG linked r-$NaYF_4$ were collected by centrifugation at 5550 g and washed with ethanol three times. Then the precipitation were re-dispersed in DMF (1 mL), and reacted with EDC·HCl (10 mg) for 30

min, afterwards DMAP (0.2 mg) and TEC (10 mg) was added into solution, vigorously stirring at room temperature for 24 h. The mixture was dialyzed against deionized water (5 kDa) to acquire r-$NaYF_4$-TEC NPs. For preparation of b-$NaYF_4$-PMB NPs, first the blue emissive $NaYF_4$:Yb,Tm nanoparticles (b-$NaYF_4$ NPs) were prepared according to the reported method.[2] The encapsulation and further functionalization procedures were similar to the mentioned above, except TEC was replaced by PMB (10 mg).

Modification of CdS@ZnS QDs and CdSe@ZnS QDs

The commercial CdS@ZnS QDs and CdSe@ZnS QDs were modified with PEG-COOH beforehand. The carboxyl-terminated CdS@ZnS QDs of red fluorescent emission were collected by centrifugation at 5550 g and washed with ethanol. Then the precipitation were dispersed in DMF (1 mL), and reacted with EDC·HCl (10 mg) for 30 min, afterwards DMAP (0.2 mg) and TEC (10 mg) was added into solution, vigorously stirring at room temperature for 24 h. The mixture was dialyzed against deionized water (5 kDa) to acquire CdS@ZnS-TEC QDs. For preparation of blue emissive CdSe@ZnS-PMB QDs, modification procedures were similar to the mentioned above, except TEC was replaced by PMB (10 mg).

Measurement of Absolute Quantum Yield of the Synthesized $NaYF_4$ NPs

The absolute quantum yield measurement was carried out on the Steady-State&Time-Resolved Fluorescence Spectrofluorometer (PTI Corporation QM/TM/IM) equipped with the integrating sphere (80 mm in diameter) from EVERFINE Corporation. The integrating sphere was mounted on the fluorescence spectrofluorometer with the entry and output ports of the sphere located in 90 geometry from each other in the plane of the spectrometer. The samples were illuminated with a 980 nm laser device (Shanghai Dream Laser Technology Co., Ltd) coupled to fiber at an excitation density of 0.5W•cm^{-2}. The spectrum of excitation radiation not absorbed by the sample was measured at the

wavelength from 970 to 1000 nm through neutral density filters. The spectrum of emission of each sample was measured from 400 to 800 nm without any neutral density filter. Samples were dispersed in ethanol, and pure ethanol was used as the reference to record blank background. The absolute quantum yield (QY) of each sample was then determined according to the equation: $QY= N_{emi} /N_{abs} \times 100\%$; where N_{abs} is absorbent excitation photon number, Here N_{abs} is corrected with background subtraction.

Synthesis of ZFO-UBI NPs

The magnetic ZFO-UBI NPs were prepared based solvothermal reaction.[3] Initially, $FeCl_3$ (3 mM), $ZnCl_2$ (1.5 mM), sodium acetate (20 mM) and sodium citrate (0.2 mM) was dissolved in ethylene glycol (20 mL) under vigorous stirring for 30 min. Following that, the resulting mixture was heated at 200 °C for 8 h. Finally, the obtained $ZnFe_2O_4$ nanoparticles (ZFO NPs) were washed with ultrapure water and ethanol alternately. Subsequently the ZFO NPs (~10 mg) were dispersed in 5 mL phosphate-buffered saline (PBS, pH = 7.4) and EDC·HCl (6 mg) was added to the solution and stirred for 2 h. Afterward, NHS (3 mg) was added and stirred at 37 °C for 10 h. The deposit was magnetically separated and re-dispersed in 5 mL PBS (pH = 7.4) containing 15 mg Ubiquicidin (29-41) then reacted at 37 °C for 24 h. The resulting ZFO-UBI NPs were separated using magnetic field and washing three times with PBS (pH = 7.4), then re-dispersed in 1 mL PBS and stored at 4 °C.

Bacteria Culture

The *Staphylococcus aureus* (ATCC 25923), and *Escherichia coli* (ATCC 25922) were inoculated in LB broth under shaking (200 rpm) at 37 °C. The bacteria concentration was determined by measuring the optical density at 600 nm (OD_{600}) via UV−Visible spectroscopy.

Qualitative Detection of Target Bacteria

S. aureus suspension (10^6 CFU/mL) or *E. coli* suspension (10^6 CFU/mL) was incubated with CaS-TEC NPs (3 mg/mL), SrS-PMB NPs (4 mg/mL) and ZFO-UBI NPs (1 mg/mL) for 60 min with gentle shaking. The bacteria-nanoparticles complex was isolated by magnetic separation, then washed with PBS（pH= 7.4, containing 0.1% Triton X-100）thoroughly. The magnetic absorbing deposits were irradiated with 980 nm laser and fluorescence images were captured by smartphone camera (Mi 9 smartphone) with NIR optical filter to remove 980 nm excitation light. In addition, bacteria mixture suspension with various concentrations (10^6 CFU/mL *S. aureus* / 10^3 CFU/m E. coli; 10^6 CFU/mL *S. aureus* / 10^6 CFU/mL *E. coli*; 10^3 CFU/mL *S. aureus* / 10^6 CFU/mL *E. coli*) were incubated with CaS-TEC NPs (3 mg/mL), SrS-PMB NPs (4 mg/mL) and ZFO-UBI NPs (1 mg/mL) for 60 min with gentle shaking, the deposits were parallel separated and imaged under 980 nm laser illumination. All the washed deposits absorbed by magnet were re-dispersed in PBS to obtain homogeneous suspensions, fluorescence spectrum of the bacteria suspensions were collected by fluorospectrophotometer, and further observed using fluorescence microscope equipped with 980 nm laser device, emission filters were set in the Cy3.5 channel and the DAPI channel for the dual-color imaging of the fluorescent AES probes tagged bacteria cell, respectively.

Quantitative Determination of Target Bacteria

Varying concentrations of *S. aureus* suspensions (25 to 10^6 CFU/mL) and *E. coli* suspensions (25 to 10^6 CFU/mL) were each incubated with CaS-TEC NPs (3 mg/mL), SrS-PMB NPs (4 mg/mL) and ZFO-UBI NPs (1 mg/mL) for 60 min with gentle shaking. The bacteria-nanoparticles complex were isolated by magnetic separation, then washed with PBS（pH= 7.4, containing 0.1% Triton X-100）thoroughly. All the washed deposits were re-dispersed in PBS to obtain homogeneous suspensions, and fluorescence signal intensity for *S. aureus* and *E. coli* determination were set at 645 nm and 465 nm, respectively.

Target Bacteria Assay in Real Samples

Milk sample and mineral water sample were purchased from supermarkets. All spiked samples were prepared by adding *S. aureus* and *E. coli* with varying known concentrations. The bacteria amount of purchased samples and spiked samples were determined and calculated by the proposed fluorescent method. For bacteria growth monitoring, milk samples were firstly sterilized and adding trace *S. aureus* (~10 CFU/mL) and *E. coli* (~10 CFU/mL), then cultured at 37 °C for 50 h. Experimental groups were parallel done except clindamycin or chloramphenicol were added.

Statistical Analysis

Bacteria determination were measured and repeated at least three times, results were given as mean ± SD. Statistical data was done with SPSS software. Significant differences were determined using a Student's t-test, and differences were set at $P<0.01$ (**).

Characterization of the AES nanoprobes and ZFO-UBI NPs

Two types of carboxylated sulfide nanoparticles (CaS NPs and SrS NPs) were synthesized and further modified with hydrophilic polyoxyethylene fragment, then teicoplanin (TEC) and polymyxin B (PMB) used as bacteria recognition ligands were covalently linked to the surface of CaS NPs and SrS NPs, respectively. Transmission electron microscopy (TEM) showed the obtained TEC modified CaS NPs (CaS-TEC NPs) and PMB modified SrS NPs (SrS-PMB NPs) were well dispersed with a mean sizes of ~ 28 nm and ~ 37 nm, respectively (Figure S1a~d). X-ray diffraction (XRD) analysis revealed the host composition of CaS-TEC NPs and SrS-PMB NPs were CaS and SrS cubic phase, see Figure S2. Zeta potential measurements showed that both the carboxylated CaS NPs and SrS NPs were negatively charged, while the zeta potential of CaS-TEC NPs became less negative due to covalent bonding of the ionized carboxyl group on the nanoparticles surface (Figure S3). The modification of amino-rich PMB molecule, which are easily protonated, made the zeta potential of SrS-PMB NPs positive (Figure S3). UV-Visible absorption band of CaS-TEC NPs and SrS-PMB NPs solution also demonstrated the existence of TEC and PMB on the nanoparticles (Figure S4). High performance liquid chromatography tandem mass spectrometry (HPLC-ESI-MS) was used to quantitatively estimate the modification of TEC and PMB. Figure S5 showed the qualitative results of TEC and PMB molecular, bonding amount of the ligands was calculated to be ~ 189 μg/mg for TEC and ~ 231 μg/mg for PMB. All the results verified successful modification of CaS NPs and SrS NPs. Further, the synthesized AES nanoprobes (CaS-TEC NPs/ SrS-PMB NPs) were proved with good stability in PBS solutions, there was no significant decline in normalized fluorescent intensity within one month storage (Figure S6).

The magnetic nanomaterials, including ZFO NPs, $NiFe_2O_4$ nanoparticles (NFO NPs) and Fe_3O_4 nanoparticles (Fe_3O_4 NPs), were prepared to evaluate their applicability in proposed magneto-fluorescent assay method, Figure S7 demonstrated the impact of these magnetic nanoparticles on fluorescent intensity of AES nanoprobes, there was negligible fluorescent quenching when AES nanoprobes were incubated with ZFO NPs, however, the fluorescence intensities were slightly affected by the NFO NPs and Fe_3O_4 NPs, it might be ascribed to the stronger absorption around 400~500 nm

than ZFO NPs, which overlapped with the emission band of SrS-PMB NPs and caused more obvious energy transfer of fluorescent nanoprobes (Figure S8). Hence the ZFO NPs were selected as magnetic capture unit and further functionalized with UBI ligands. Characterization of ZFO NPs and ZFO-UBI NPs were presented in Figure 2 and Figure S9, S10.

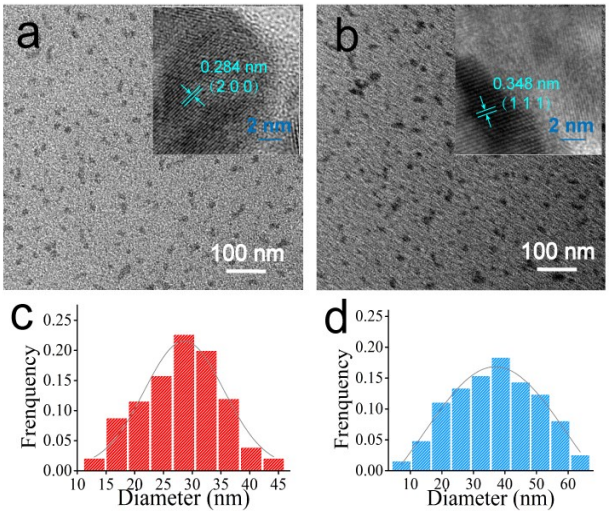

Figure S1. TEM and (inset) HRTEM of: (a) CaS-TEC NPs, (b) SrS-PMB NPs. And corresponding particle size histogram of: (c) CaS-TEC NPs, (d) SrS-PMB NPs.

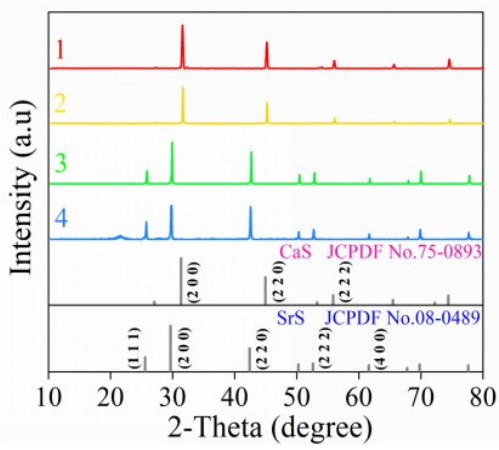

Figure S2. XRD of the CaS NPs (1), CaS-TEC NPs (2), SrS NPs (3) and SrS-PMB NPs (4).

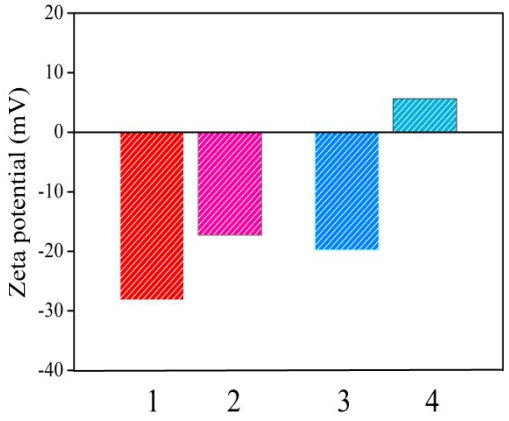

Figure S3. Zeta potential of the CaS NPs (1) and CaS-TEC NPs (2), SrS NPs (3) and SrS-PMB NPs (4).

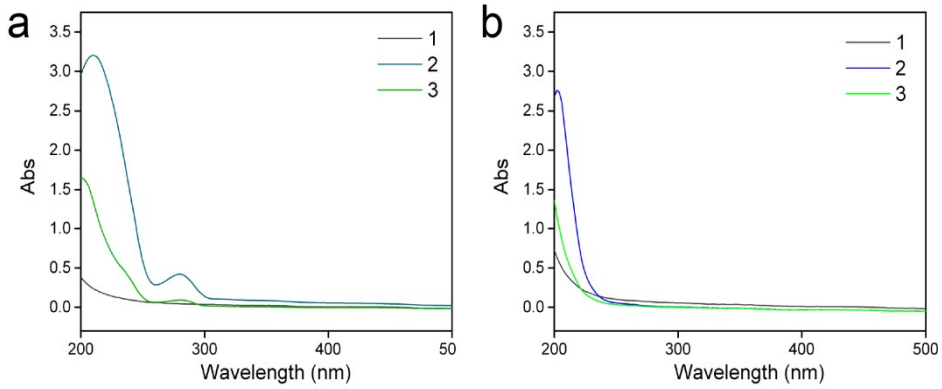

Figure S4. UV spectrum of the (a) CaS NPs (1), TEC (2) and CaS-TEC NPs (3), and the UV spectrum of the (b) SrS NPs (1), PMB (2) and SrS-PMB NPs (3).

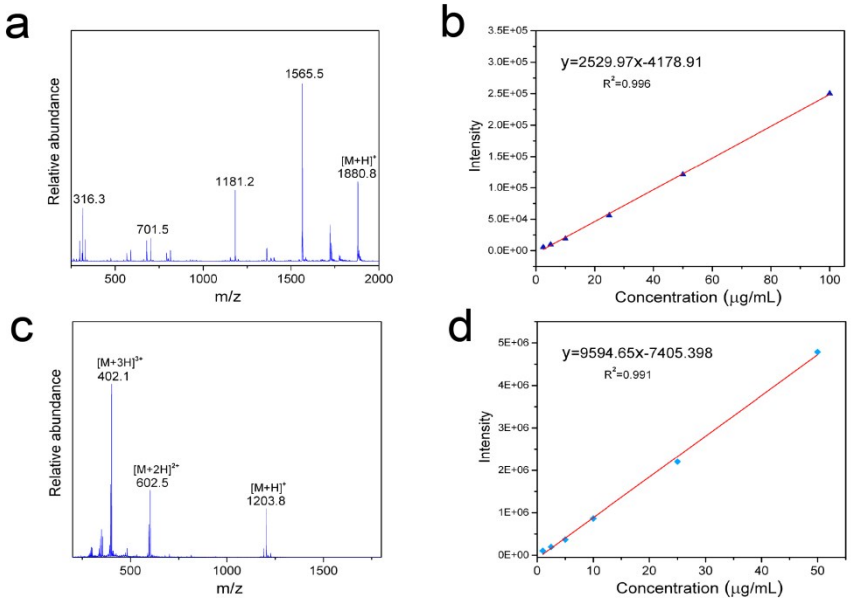

Figure S5. MS spectrum of the (a) TEC and (c) PMB, and the calibration curve of the (b) TEC and (d) PMB standard solutions.

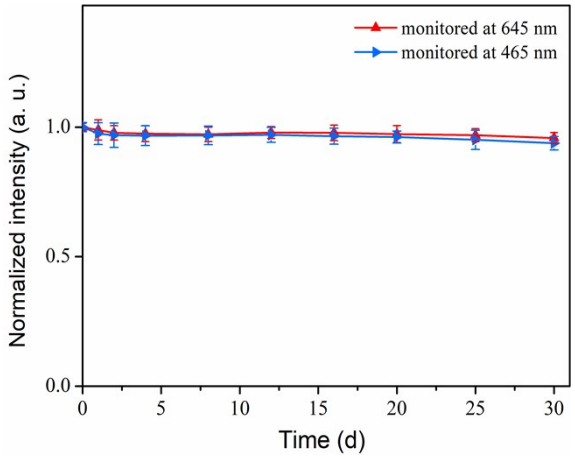

Figure S6. Normalized fluorescent intensity of AES nanoprobes after one month storage in PBS solution (pH=7.4).

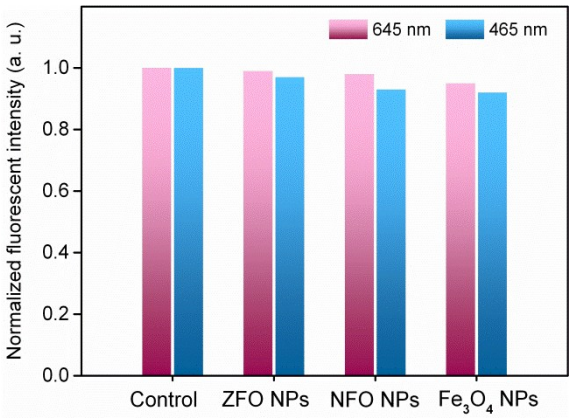

Figure S7. Fluorescent intensity of AES nanoprobes mixed with the three different magnetic nanoparticles solutions (1mg/mL).

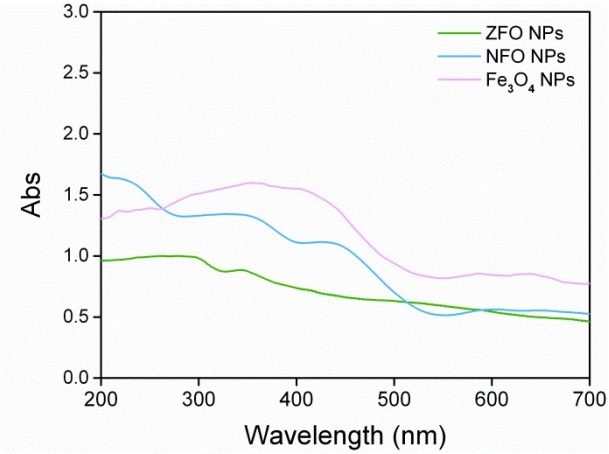

Figure S8. UV-visible spectrum of the three different magnetic nanoparticles solutions (1mg/mL).

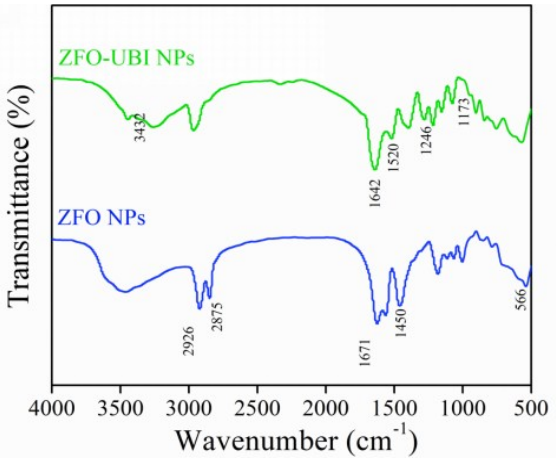

Figure S9. Fourier transform infrared spectrum of the synthesized ZFO NPs and ZFO-UBI NPs.

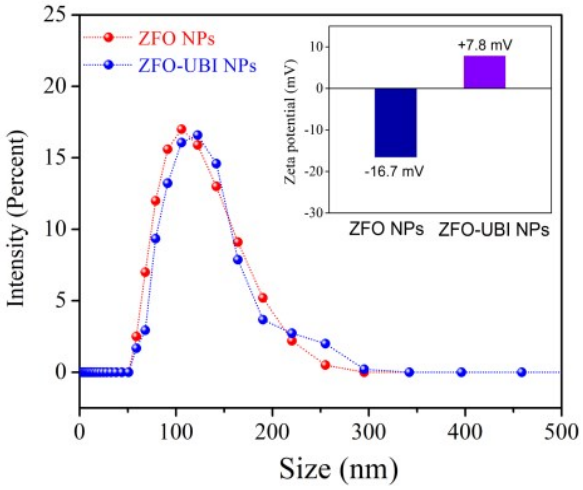

Figure S10. DLS and Zeta potential (inset) of the synthesized ZFO NPs and ZFO-UBI NPs.

Supplemental Figures

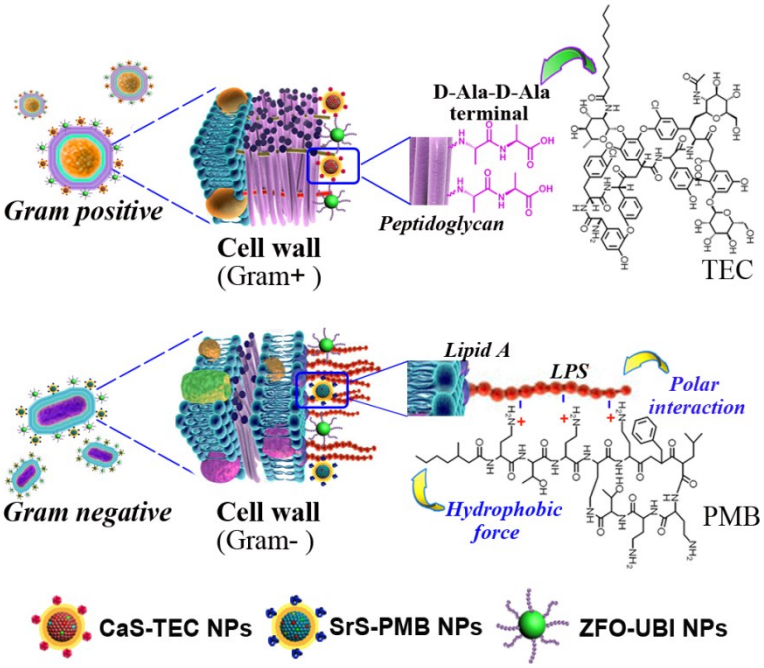

Figure S11. Schematic diagram illustrating the mechanism of the AES nanoprobes targeted to Gram positive or negative bacteria cell.

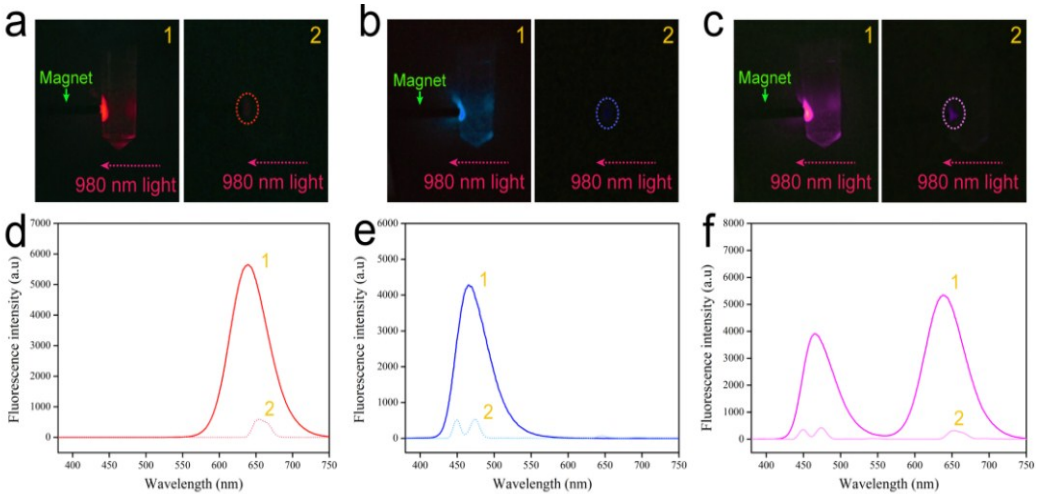

Figure S12. NIR irradiated fluorescence images of magnetically separated and washed precipitation of (a) *S. aureus* suspension, (b) *E. coli* suspension, and (c) *S. aureus/E. coli* mixture suspension incubated with AES nanoprobes and ZFO-UBI NPs (1), or incubated with $NaYF_4$ NPs and ZFO-UBI NPs (2).

Corresponding precipitations in (a)~(c) are redispersed and the 980 nm light excited fluorescence spectrum (d)~(f) are collected, respectively.

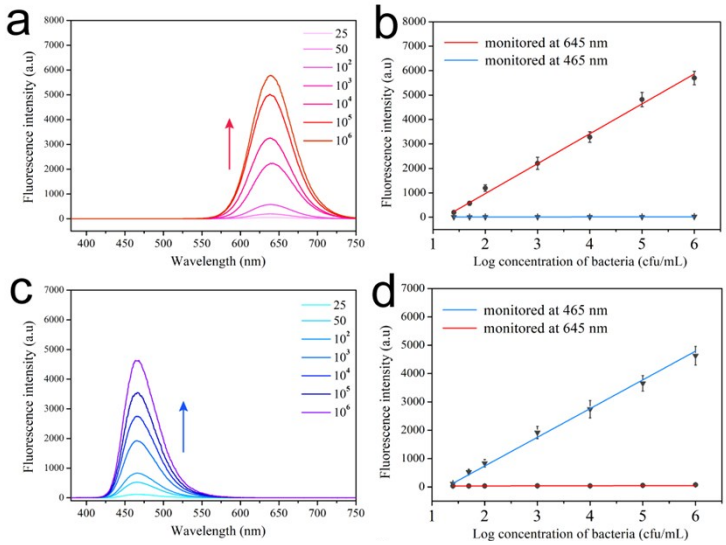

Figure S13. NIR (980 nm) excited fluorescence spectrum and the linear curve of the fluorescence intensity versus the bacteria concentration of: (a, b) *S. aureus* suspension, (c, d) *E. coli* suspension, respectively.

Table S1. Experimental absolute quantum yields of the synthesized r-/b-$NaYF_4$ NPs.

Compositions	Emissive photon number	Absorbent photon number	QY [%]
r-$NaYF_4$ NPs	6952	1782473	~0.39
b-$NaYF_4$ NPs	8521	1852390	~0.46

Table S2. Corresponding CIE color coordinate value of the five bacteria precipitation samples.

Sample No.	Coordinate value
1	(0.6812, 0.3185)
2	(0.4955, 0.2570)
3	(0.3799, 0.2197)
4	(0.2484, 0.1734)
5	(0.1295, 0.1341)

Table S3. The L_{16} (4^3) orthogonal table to optimize the *S. aureus* determination and corresponding fluorescence intensity results under orthogonal experimental conditions

Serial No.	CaS-TEC NPs (mg/mL)	ZFO-UBI NPs (mg/mL)	Incubation time (min)	$F_{645\ nm}$ (a.u)
Exp. 1	1	0.25	30	1265
Exp. 2	1	0.5	60	1631
Exp. 3	1	1	120	1643
Exp. 4	1	2	180	1398
Exp.5	2	0.25	60	3095
Exp. 6	2	0.5	30	2743
Exp. 7	2	1	180	3673
Exp. 8	2	2	120	3504
Exp. 9	3	0.25	120	4013
Exp. 10	3	0.5	180	4332
Exp. 12	3	1	30	4689
Exp. 13	3	2	60	4467
Exp. 14	4	0.25	180	3960
Exp. 15	4	0.5	120	4124
Exp. 16	4	1	60	4503
Mean-1	1484	3083	3177	
Mean-2	3254	3208	3424	
Mean-3	4375	3627	3321	
Mean-4	4150	3345	3341	
Extremum	2891	543.75	246.75	

Table S4. The L_{16} (4^3) orthogonal table to optimize the *E. coli* determination and corresponding fluorescence intensity results under orthogonal experimental conditions

Serial No.	SrS-PMB NPs (mg/mL)	ZFO-UBI NPs (mg/mL)	Incubation time (min)	$F_{465\ nm}$ (a.u)

Exp. 1	1	0.25	30	683
Exp. 2	1	0.5	60	925
Exp. 3	1	1	120	1195
Exp. 4	1	2	180	1341
Exp.5	2	0.25	60	1437
Exp. 6	2	0.5	30	1568
Exp. 7	2	1	180	1890
Exp. 8	2	2	120	1932
Exp. 9	3	0.25	120	2851
Exp. 10	3	0.5	180	3028
Exp. 12	3	1	30	3209
Exp. 13	3	2	60	3312
Exp. 14	4	0.25	180	2897
Exp. 15	4	0.5	120	3341
Exp. 16	4	1	60	3656
Mean-1	1036	1967	2192	
Mean-2	1707	2216	2333	
Mean-3	3100	2488	2330	
Mean-4	3300	2473	2289	
Extremum	2264	521	141	

Table S5. Comparison of the analytical performance of other bacteria assays.

Detection methods	Linear range (CFU/mL)	LOD (CFU/mL)	Bacterium	Probes	Ref
Fluorescence	32-10^8	16	*S. aureus*	Au	4
Fluorescence	10^2-10^7	50.2	*Desulfotomaculum*	NIR-HS	5
Colorimetry	1.5×10^2-1.5×10^6	1.5×10^3	*S. aureus*	Au	6
Colorimetry	10^3-10^6	41	*E.coli*	AuNPs	7
Chemiluminescence	10^4–10^7	28.8×10^3	*Salmonella*	Phenoxy-dioxetane	8

Electrochemical	$10\text{-}10^6$	1	*S. aureus*	Ag NPs	9
Microfluidic Device	/	10	*E. coli*	Fe_3O_4 nanoparticles	10
SPR	$10^3\text{-}10^6$	10^2	*E.coli*	Fe_3O_4@Au microsphere	11
SERS	$10^3\text{-}10^7$	10^3	*E.coli*	Gold nanohole array	12

Table S6. Recovery efficiency of *S. aureus* and *E. coli* spiked in the samples based on the proposed assay strategy

Samples	Measure value (Log CFU/mL)		Spiked value (Log CFU/mL)		Found value (Log CFU/mL)		Recovery (%)		RSD (%)	
	S.aureus	*E.coli*	*S.aureus*	*E.coli*	*S.aureus*	*E.coli*	*S.aureus*	*E.coli*	*S.aureus*	*E.coli*
Milk 1	/	/	1.29	1.18	1.39	1.26	107.8	106.8	8.2 %	7.9 %
Milk 2	/	/	2.56	2.37	2.69	2.43	105.1	102.5	5.9 %	6.5 %
Milk 3	/	/	5.37	5.06	5.41	5.08	100.7	100.4	6.3 %	6.0 %
Mineral water 1	/	/	1.29	1.18	1.32	1.16	102.3	98.3	4.1 %	4.7 %
Mineral water 2	/	/	2.56	2.37	2.66	2.43	103.9	102.5	5.9 %	3.9 %
Mineral water 3	/	/	5.37	5.06	5.43	5.03	101.1	99.4	3.3 %	4.7 %

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