Electronic Supplementary Material (ESI) for Analyst.

Upconverting Ion-Selective Nanoparticles for the imaging of Intracellular Calcium Ions

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Experiential Section.

Chemicals and Cell Culture. Oil dispersible upconverting nanoparticles (UCNPs, ~ 25 nm) were obtained from Hefei Fluonano Biotech Co. (Hefei, China). Compound 6 (P6) was synthesized as reported.[S1] All the other chemicals were purchased from Sigma-Aldrich. All aqueous solutions were prepared using ultrapure water (Milli-Q, Millipore).

HeLa cells (Institute of Biochemistry and Cell Biology, Shanghai Institute for Biological Sciences) were cultured at 37℃ in a humidified incubator with 5% CO_2. The medium used was high glucose Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and 1% antibiotics (penicillin/streptomycin).

Preparation of upconverting calcium-selective nanoparticles. Nanoparticles were prepared by a precipitation method as reported.[S2,S3] 1.9 mg of NaTFPB, 0.48 mg of P6, 3.9 mg of calcium ionophore Ⅱ, 16 mg of DOS, and 10 mg of F127 were dissolved in 3.0 mL of tetrahydrofuran (THF) to form a homogeneous solution. Then, 10 μL of cyclohexane solution with UCNPs (8 mg/mL) and 0.5 mL of the prepared solution were injected into 4.5 mL of ultrapure water and spinned with a speed of 1000 r/min to form the nanoparticles. Finally, the resulting clear mixture containing nanoparticles was blown with N_2 for at least 30 min to remove THF.

Characterization of nanoparticles. The size and morphology of synthesized nanoparticles were recorded by transmission electron microscopy (TEM) with a JEOL model 2000 instrument operated at acceleration voltage of 200 kV. The adsorption spectrum of nanoparticles in the solution was measured by a Nonodrop-2000C spectrophotometer. The fluorescence spectrum of nanoparticles was determined using a Hitachi fluorescence spectrometer. Up-conversion fluorescence spectra was determined using a ZolixScan ZLX-UPL spectrometer with an external 1.5 W continuous-wave laser (980 nm) as the excitation source.

MTT Assay. The cytotoxicity of the upconverting ion-selective nanoparticles was detected by MTT assay. The HeLa cells with density of 1×10^4 cells/well were incubated in a 96-well plate (200 μL/well) overnight. Then the nanoparticles of different concentrations were added into wells and cultured in the medium for 1 h in sequence. The cell seeded in wells with pure culture medium was used for the control group. 50 μL of MTT solution (5 mg/mL) was added into each well in the dark. The MTT solution was removed after 4 h. Afterward, the dimethyl sulfoxide (150 μL/well) was added to solubilize the formazan crystals. The absorbance was measured at

570 nm by the Enspire multimode plate reader, and the cellular viability was calculated from the following formula:

$$viability\ (\%) = \frac{OD_{test} - OD_{blank}}{OD_{control} - OD_{blank}} \times 100$$

Imaging of intracellular calcium ions in cells. HeLa cells were cultured on confocal dishes in high glucose DMEM for 24 h prior to the measurement. The nanoparticles suspensions (0.5 mL) were cultured with the cells at 37℃ overnight. Fluorescence imaging was performed using an inverted fluorescence microscope (Nikon Ti, Japan) and a filter set (LL01-980-25, FF930-SDi01 and FF01-890/SP). During the imaging process, ionomycin (5 μM) was used to raise the concentration of intracellular calcium.

Figure S1. (A) The fluorescence spectrum of UCNPs under the excitation of 980 nm; (B) the adsorption spectrum of P6 in the solutions with different pHs .

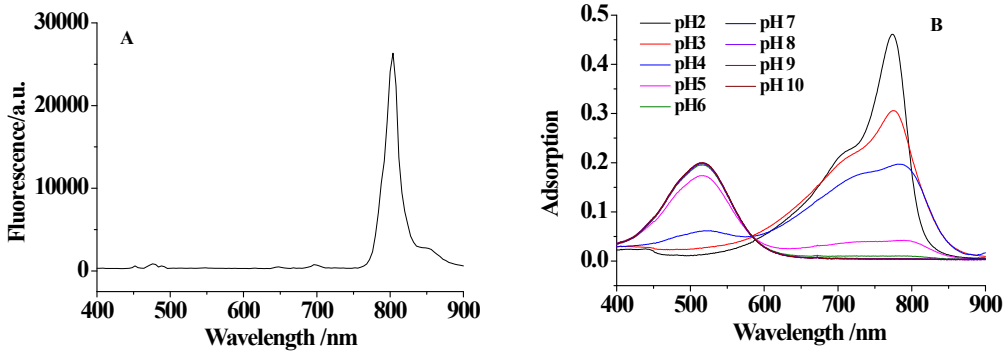


Figure S2. The adsorption spectrums of upconverting calcium-selective nanoparticles in presence of aqueous calcium ions from 10^{-8} to 10^{-1} M (inset: the solutions in absence and presence of 0.1 M calcium ions).

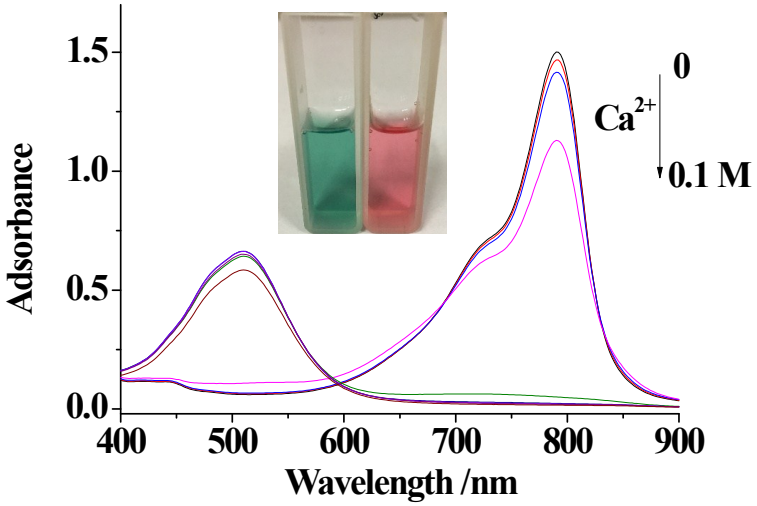


Figure S3. The selective responses of upconverting calcium-selective nanoparticles to calcium ions and the interfering ions. The concentrations of all these ions are 1 mM. The fluorescence intensity of the nanoparticles to calcium ions is defined as 100%.

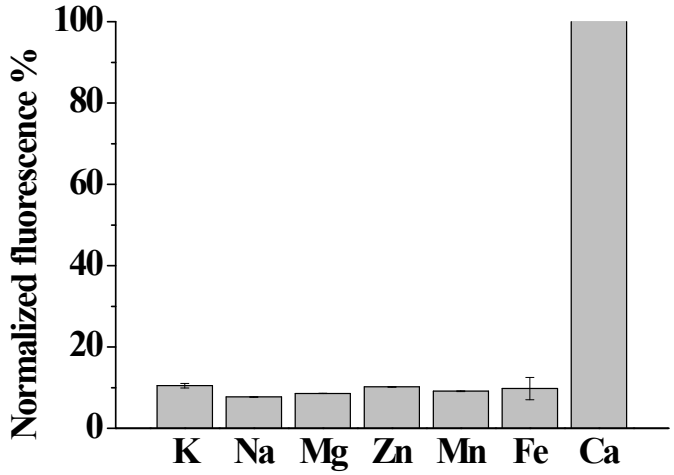


Figure S4. The fluorescence emission spectrum from the lysed cells preloaded with the nanoparticles.

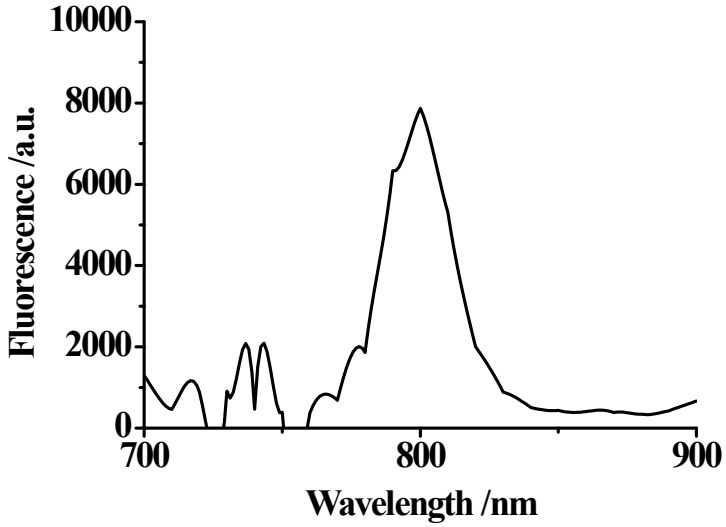
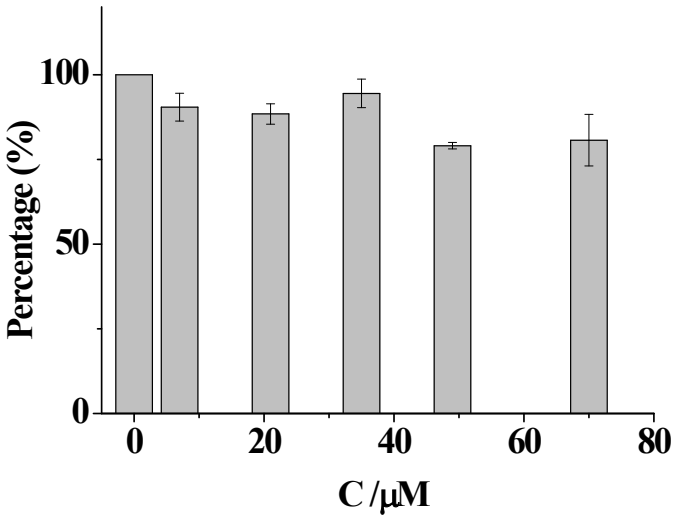


Figure S5. MTT assay to investigate the cytotoxicity of these nanoparticles at the cells. The nanoparticles with different concentrations in HeLa cells (1×10^4 cells/well) cultured for 1 h at 37℃.The error bar presented the standard deviation from three measurements.



Reference.

S1. J. R. Hou, D. Jin, B. Chen, L. L. Si, Y. H. Jin, L. G. Chen, X. L. Yan, B. W. Wang and Y. Li, *Chinese Chem. Lett.*, 2017, **28**, 1681-1687.

S2. S. Zhou, X. Peng, H. Xu, Y. Qin, D. Jiang, J. Qu and H. Y. Chen, *Anal. Chem.*, 2018, **90**, 7982-7988.

S3. X. Xie, J. Zhai and E. Bakker, *Anal. Chem.*, 2014, **86**, 2853-2856.