Electronic Supplementary Material

Construction of a novel fluorescent nanoenzyme based on lanthanides for tumor theranostics

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1 Characterization

Crystal structures of as-prepared samples were characterized using a powder X-ray diffraction (XRD) apparatus (D/Max 2500). Morphologies and element compositions of samples were characterized through transmission electron microscopy (TEM) and high-resolution transmission electron microscopy (HRTEM) using a JEOL JEM-2100 microscope equipped with an Oxford instrument energy dispersive X-ray spectroscopy (EDS) system at the accelerating voltage of 200 kV. Upconversion emission spectra were recorded by a fluorescence spectrophotometer (R500) under the excitation of a 980 nm laser. Upconversion optical bioimaging was performed under a laser scanning confocal microscope (ZEISS710). *In vivo* upconversion luminescence (UCL) imaging was performed through a *in vivo* imaging system (Berthold Technologies: NightOWL LB983).

2 Characterization of peroxidase-mimicking catalytic activity

The peroxidase-mimicking catalytic activity of ligand-free $Ba_{1.4}Mn_{0.6}LuF_7$: $Yb^{3+}/Er^{3+}/Ho^{3+}$ (BMLF) was studied through 3, 3′, 5, 5′-tetramethylbenzidine (TMB). The steady-state kinetic assays were performed in 4 mL of the phosphate buffer saline (PBS) solution (pH 4.0) with BMLF (100 $\mu g \cdot mL^{-1}$) as a catalyst in the presence of H_2O_2 (100 $\mu mol \cdot L^{-1}$) and TMB (0.2 $mmol \cdot L^{-1}$). In detail, 400 μg of BMLF was added into 4 mL of acetate buffer (0.1 $mol \cdot L^{-1}$, pH 4.0) containing 0.2 $mmol \cdot L^{-1}$ of TMB followed by the addition of H_2O_2 (4 μL, 0.01 $mol \cdot L^{-1}$). After a certain reaction time, the absorbance (655 nm characteristic peaks) of color reactions was measured through an ultraviolet–visible (UV–vis) spectrophotometer. Notably, TMB needs to be dissolved in dimethyl sulfoxide (DMSO), which was then added into acetate buffer.

3 Biological experiments

3.1 Cell culture

HeLa, CT26, and mouse embryonic fibroblast (MEF) cells, purchased from BeNa Culture Collection, were grown in the medium, Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS, GIBCO), fungizone, L-glutamine, streptomycin, and penicillin. These cells were seeded in 24-well glass bottom plate at a density of 10 000, and incubated at 37 °C for 72–96 h in 95% air plus 5% CO_2 in order to allow them to reach ~90% confluence. The medium was changed every 2 d.

3.2 Cytotoxicity assays

The effect of BMLF on cell viabilities of MEF and CT26 cells was determined using the 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2-H-tetrazolium bromide (MTT) assay. The cell culture medium in each well was then replaced by 200 μL of cell growth medium containing BMLF with different concentrations (200, 400, 600, 800, and 1000 $\mu g \cdot mL^{-1}$). After incubation for 24 h, 200 μL of MTT (0.5 $mg \cdot mL^{-1}$ in PBS) was added into each well for another 4 h at 37 °C. Subsequently the grown medium was removed gently with the aid of suction followed by the addition of 300 μL of DMSO to each well as a solubilizing agent. The microplate was at last left at room temperature for 2 h.

3.3 Intracellular reactive oxygen species (ROS) detection

CT26 cells were seeded into a confocal dish at a density of 4×10^5 cells/well and incubated for 24 h at 37 °C under a humidified atmosphere with 5% CO_2. The cells were then co-incubated with BMLF (100 $\mu g \cdot mL^{-1}$) or PBS for 4 h. After they were washed with PBS three times, each plate was incubated with 0.5 μL DCFH-DA in 1 mL of serum-free RPMI at 37 °C for 20 min in the dark and mixed evenly every 3–5 min. Finally, fluorescence signals of DCF inside CT26 cells were recorded using laser scanning confocal microscopy (LSCM).

3.4 UCL imaging *in vitro*

HeLa cells were stained with BMLF and then imaged with confocal microscopy. Firstly, BMLF was dispersed in PBS (200 $\mu g \cdot mL^{-1}$) and diluted in culture medium of 10 μL for 20 min at 37 °C in 95% air plus 5% CO_2. Subsequently, cells were washed with PBS for four times to remove excessive BMLF. Afterwards, the UCL imaging of living cells was visualized under a laser scanning confocal microscope (Leica TCS SP5) with a laser excitation source of 980 nm. Finally, UCL signals were separately collected in the channels of 520–570 nm and 630–670 nm.

3.5 Animals and tumor models

BALB/c mice, female, 4 weeks of age, about 15–20 g, were purchased from Anhui Medical University. All animal experiments were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Anqing Normal University and approved by the Animal Ethics Committee of Anqing Normal University. CT26 tumor models were established through subcutaneously injecting CT26 cells (2×10^6 cells suspended in 100 μL of PBS buffer) into the right abdomen of each mouse. When the tumor volume reached ~100 mm^3, *in vivo* experiments were implemented.

3.6 UCL imaging for tumor *in vivo*

The BMLF aqueous suspension (0.1 mL, 200 μg·mL^{-1}) was intravenously administrated into the colon cancer-bearing mice. The UCL imaging experiments were performed after ~1 h post-injection of BMLF through the *in vivo* imaging system.

4 EDS and XPS results of BMLF

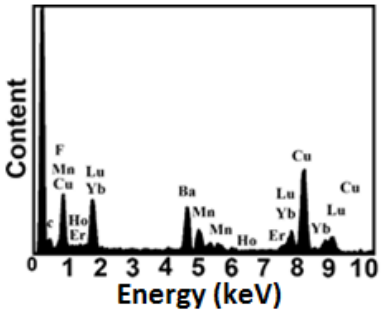

Fig. S1 EDS spectrum of BMLF.

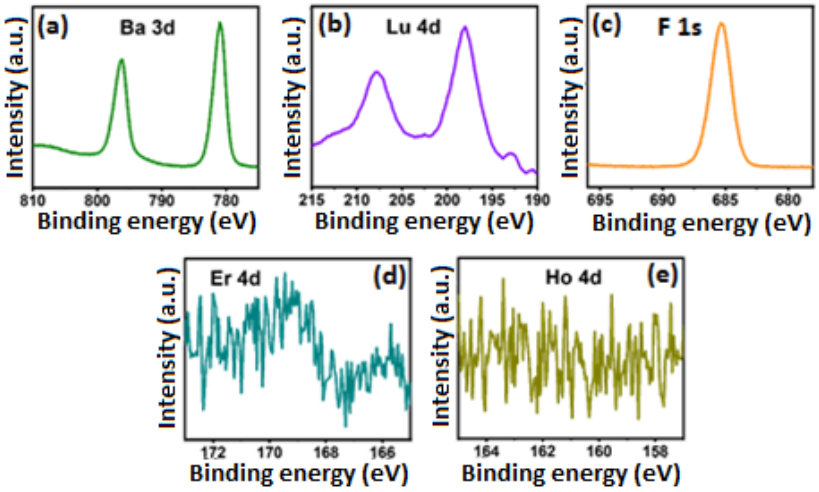

Fig. S2 High-resolution XPS patterns of BMLF: **(a)** Ba 3d; **(b)** Lu 4d; **(c)** F 1s; **(d)** Er 4d; **(e)** Ho 4d.

5 Chromaticity coordinates of BMLF in the H_2O_2 solution

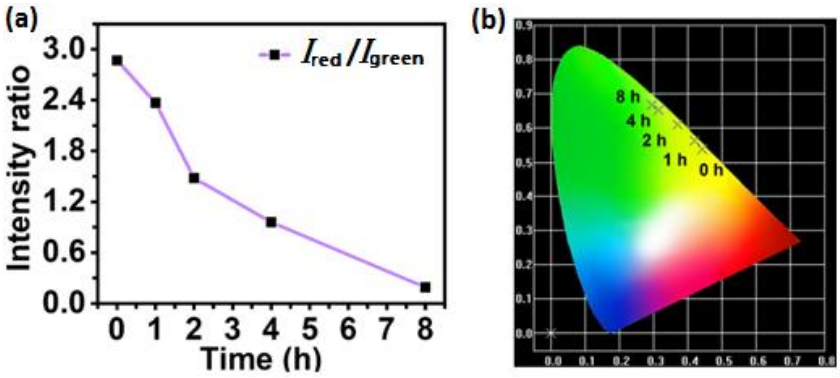

Fig. S3 (a) Variation of I_{red}/I_{green} with time. **(b)** Chromaticity coordinates of BMLF in the H_2O_2 solution with different time points.

6 *In vivo* UCL imaging of tumor-bearing mice after 10 h

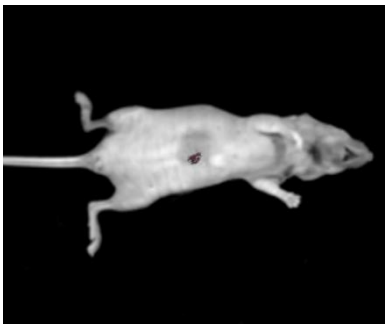

Fig. S4 *In vivo* UCL image of a tumor-bearing mouse with the intravenous injection of BMLF for 10 h at the tumor area (corresponding emission filter: 600–700 nm).

7 LSCM images of cancer cells stained with BMLF

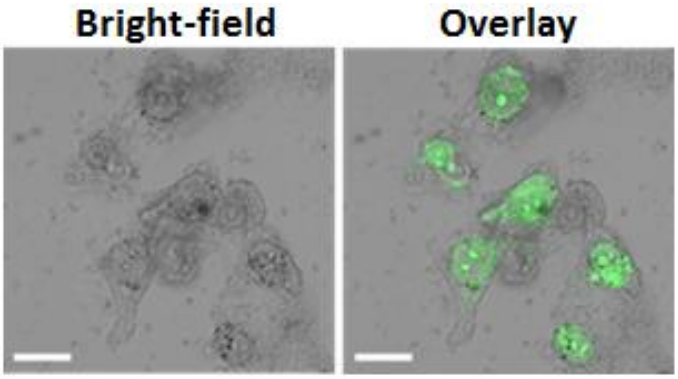

Fig. S5 LSCM images of cancer cells stained with BMLF and DCFH-DA corresponding to bright-field (left) and overlay (right) (scale bar = 20 μm).

8 Corresponding interactive 3D surface plots of cancer cells with DCFH-DA

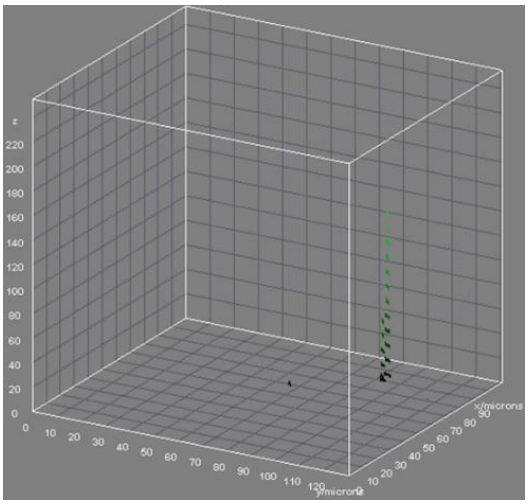

Fig. S6 Corresponding interactive 3D surface plots of cancer cells stained with DCFH-DA.