

Electronic Supplementary Information for

Extending the excitation wavelength from UV to visible light for a europium complex-based mitochondria targetable luminescence probe for singlet oxygen

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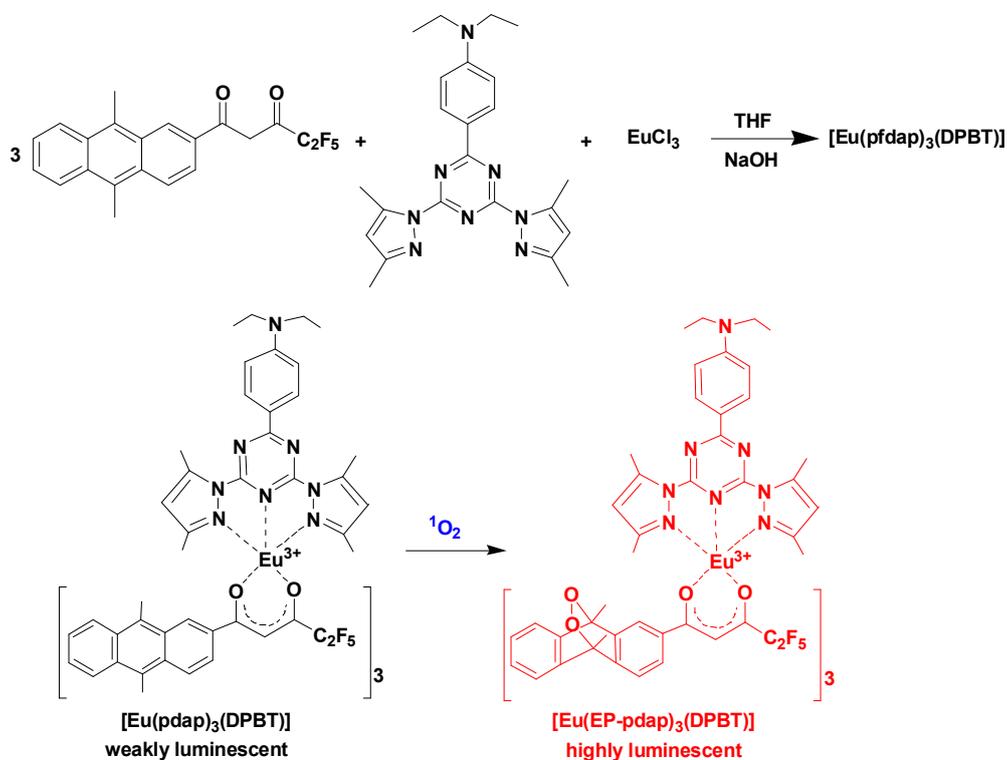
1. Materials and physical measurements

The compound 1,1,1,2,2-pentafluoro-5-(9',10'-dimethyl-2'-anthryl)-3,5-pentanedione (pdap) and 2-(N,N-diethylanilin-4-yl)-4,6-bis(3,5-dimethylpyrazol-1-yl)-1,3,5-triazine (DPBT) was synthesized according to the literature method.^{1, 2} Europium (III) Chloride Hexahydrate ($\text{EuCl}_3 \cdot 6\text{H}_2\text{O}$), 3-(4,5-dimethyl-2-thiazol-yl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) , Horseradish peroxidase (HRP, RZ = 2.5, >200 U/mg) and 3-indoleacetic acid (IAA) were purchased from Aladdin. Tetrahydrofuran (THF) was used after appropriate distillation and purification. Triton X-100 was purchased from Acros Organics. Mito Tracker Green FM were purchased from Shanghai Yeasen biological technology co., LTD (China). Chlorin e6 was purchased from J&K Scientific. MCF-7 cells were obtained from School of pharmaceutical science and technology, Dalian University of Technology. H22-tumor bearing BALB/c mice were obtained from Dalian Medical University. *Daphnia magna* was obtained from Professor Jingwen Chen's group at School of Environmental Science and Technology, Dalian University of Technology. 5-Aminolevulinic acid (ALA) was purchased from Shanghai Fudan-Zhangjiang Bio-Pharmaceutical Co. Ltd. (China). 5,10,15,20-Tetrakis(1-methyl-4-pyridinio)porphyrin tetra(*p*-toluenesulfonate) (TMPyP) was purchased from Fluka Chemistry (Switzerland). Unless otherwise stated, all chemical materials were purchased from commercial sources and used without further purification. All living cells, living mouse experiments were performed in compliance with the relevant laws and institutional guidelines, and also the institutional committee of Dalian University of technology has approved the experiments.

¹H NMR spectra were measured on a Bruker Avance spectrometer (400 MHz). Mass spectra were measured on an Agilent 6130MSD MS spectrometer. Elemental analysis was carried out on a Vario-EL CHN analyser. Time-gated luminescence spectra were measured on a Perkin-Elmer LS 50B luminescence spectrometer with the settings of delay time, 0.2 ms; gate time, 0.4 ms; cycle time, 20 ms; excitation slit, 10 nm; and emission slit, 10 nm. Luminescence lifetimes were measured on a FS5 Fluorescence Spectrometer of Edinburgh Instruments. Bright-field and luminescence imaging measurements were carried out on a laboratory-use true color time-gated luminescence microscope.³ The time-gated luminescence imaging measurements were carried out with the conditions of delay time, 33 ms; gate time, 1.0 ms; lamp pulse width, 80 ms; and exposure time, 1s. The confocal fluorescence imaging measurements were carried out on a

olympus FV1000 confocal laser scanning microscope.

2. Synthesis of the Eu^{3+} complex $[\text{Eu}(\text{pdap})_3(\text{DPBT})]$ and its reaction with $^1\text{O}_2$



Scheme S1. Synthetic route of $[\text{Eu}(\text{pdap})_3(\text{DPBT})]$ and its luminescence response reaction with $^1\text{O}_2$.

2.1 Synthesis of $[\text{Eu}(\text{pdap})_3(\text{DPBT})]$

To 10 mL of THF was added 177 mg Hpdap (0.45 mmol), 55 mg $\text{EuCl}_3 \cdot 6\text{H}_2\text{O}$ (0.15 mmol), 62 mg DPBT (0.15 mmol) and 0.45 mL of aqueous solution of 1.0 M NaOH with stirring. After the mixture was refluxed for 2 h under an argon atmosphere, the solution was cooling to room temperature. After the mixture was filtered, the resulting filtrate was evaporated to remove THF, and the crude product was washed with water and n-hexane to give the target complex as dark-yellow powder. (145mg, 55.2% yield). ^1H NMR (400 MHz, DMSO-d_6): δ (ppm) = 1.16 (6H), 2.27 (7H), 2.73-2.91 (16H), 2.93-3.15 (3H), 3.46 (4H), 4.49 (2H), 6.27 (2H), 6.82 (2H), 7.26 (3H), 7.52 (7H), 7.80 (3H), 8.01 (3H), 8.11 (3H), 8.26-8.31 (5H). ESI-MS (m/z): 1771 ($[\text{M}+\text{Na}]^+$). Elemental analysis calcd (%) for $\text{C}_{86}\text{H}_{70}\text{EuF}_{15}\text{N}_8\text{O}_6$ ($[\text{Eu}(\text{pdap})_3(\text{DPBT})]$): C 59.08, H 4.04, N 6.41; found (%): C 59.48, H 3.80, N 6.20.

2.2 Reaction of [Eu(pdap)₃(DPBT)] with ¹O₂

The reaction of [Eu(pdap)₃(DPBT)] with ¹O₂ in aqueous media was investigated by using the Na₂MoO₄-H₂O₂ system as ¹O₂ source. The experimental details are described as follows. The reaction was carried out in 0.1 M carbonated buffer of pH 10.5 containing 50 μM [Eu(pdap)₃(DPBT)], 50 mM Na₂MoO₄ and 1% Triton X-100. After different concentrations of H₂O₂ were added, the solutions were stirred for 3 h at room temperature and 10-fold diluted with 0.05 M borate buffer of pH 7.4, and then the time-gated luminescence spectra were measured.

2.3 Reactions of [Eu(pdap)₃(DPBT)] with various ROS/RNS

All the reactions (except ¹O₂ was generated in alkaline media) were carried out in 0.05 M Tris-HCl buffer of pH 7.4 containing 1% Triton X-100 with the same concentrations of [Eu(pdap)₃(DPBT)] (50 μM) and ROS/RNS (0.1 mM) for 3 h at room temperature. After the reaction, the solutions were 10-fold diluted with 0.05 M borate buffer of pH 7.4, and then their time-gated emission intensities at 607 nm were recorded. Hydrogen peroxide was diluted immediately from a stabilized 30% solution. The stock solution of ClO⁻ was prepared from the commercial solution of sodium hypochlorite. Hydroxyl radical ([•]OH) was generated from the (NH₄)₂Fe(SO₄)₂-H₂O₂ system. Nitric oxide (NO) was generated by using NOC-13 as a donor. Peroxynitrite (ONOO⁻) was generated by using 3-morpholinopyridone (SIN-1) as a donor.

3. MTT assay of [Eu(pdap)₃(DPBT)] to MCF-7 cells

The cytotoxicity of [Eu(pdap)₃(DPBT)] to MCF-7 cells was measured by standard MTT assay using the previous reported method. MCF-7 cells, cultured in Dulbecco's modified Eagle's medium (DMEM, Invitrogen) supplemented with 10% fetal bovine serum, antibiotics (100 units/mL penicillin and 100 mg/mL streptomycin) were seeded in a 96-well tissue culture plate at a density of 10⁵ cells/well. After incubated for 24 h at 37 °C in a humidified 5% CO₂/95% air incubator, different concentrations of [Eu(pdap)₃(DPBT)] were added and the cells were incubated for another 24 h at the same condition. Then the cells were washing thrice with PBS and further incubated with PBS (137 mM NaCl, 2.7 mM KCl, 10.1 mM Na₂HPO₄ and 1.8 mM KH₂PO₄, pH 7.4) containing 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) for 4h. After removing the supernatant, the cells were dissolved with DMSO and then the absorbance at 540 nm was measured.

4. TGL imaging of ¹O₂ in live MCF-7 cells

The MCF-7 cells cultured in 20-mm glass bottom culture dishes were incubated with 1.0 mL of DMEM medium containing 30 μM ALA for 8 h. After washing thrice with the isotonic saline solution (140 mM NaCl, 10 mM glucose and 3.5 mM KCl), the cells were further incubated with [Eu(pdap)₃(DPBT)] (10 μM) in 1.0 mL of the isotonic saline solution containing 0.5% DMSO and 0.1% Triton X-100 for 40 min at 37 °C in the incubator. The obtained cells were irradiated for different time under a 660 nm LED lamp, and then subjected to the time-gated luminescence imaging measurements on the microscope (excitation filter, 400 \pm 20 nm). In the control group, the MCF-7 cells were first incubated with 30 μM ALA in culture media for 8h, followed by incubated with [Eu(pdap)₃(DPBT)] (10 μM) and NaN₃ (200 μM) for 1 h. The steady-state luminescence images and time-gated luminescence images were recorded on the microscope with an exposure time of 1s.

5. Colocalization imaging of [Eu(pdap)₃(DPBT)] and MitoTracker Green FM in MCF-7 cells

MCF-7 cells cultured in 20-mm glass bottom culture dishes were first incubated with 1 mL of DMEM medium containing 20 μM IAA for 0.5 h. After washing thrice with isotonic saline solution, the cells were further incubated with [Eu(pdap)₃(DPBT)] (10 μM) and HRP (0.5 μM) in 1.0 mL of 0.01 M acetate buffer (pH 4.0) containing 0.5% DMSO and 0.1% Triton X-100 for 1h at 37 °C and then the cells were further incubated with Mito Tracker Green FM (100 nM in PBS) for another 0.5 h. After washing with PBS, the cells were subjected to the confocal fluorescence imaging measurements on the confocal laser scanning microscope.²

6. Luminescence imaging of ¹O₂ in frozen tumor tissue slices of a mouse

The frozen tumor slices were prepared from the H22-tumor bearing BALB/c mouse. In detail, the subcutaneous tumor allograft model of BALB/c mouse (with a weight of \sim 20 g) implanting H22 cells were obtained. The tumor tissue was separated from the body and frozen for 24 h. Then the frozen tumor tissue was cryosectioned by using a microtome at -20 °C into slices of 15 μm thicknesses. The slices were incubated with [Eu(pdap)₃(DPBT)] (10 μM) and Chlorin e6 (10 μM) in PBS for 1h at 37 °C. The slices were washed with PBS three times. The tissues were irradiated for 10 min under a 660 nm LED lamp and then time-gated luminescence imaging were collected on the microscope (excitation filter, 400 \pm 20 nm).

7. Luminescence imaging of ¹O₂ in *D. magna*

Daphnia magna (age < 48 h), cultured in nonchlorinated tap water at 20 °C under cool-white fluorescence light with a 14:10 h light:dark photoperiod, were incubated with [Eu(pdap)₃(DPBT)] (10 μM) and TMPyP (10 μM) in nonchlorinated tap water containing 0.1% Triton X-100 for 1 h. After washing, the *D. magna* were further irradiated for 0.5 h under a 660 nm LED lamp and then time-gated luminescence imaging were collected on the microscope (excitation filter, 400±20 nm; exposure time, 100 ms). In the control group, the *D. magna* were incubated with [Eu(pdap)₃(DPBT)] (10 μM), TMPyP (10 μM) and NaN₃ (200 μM) in culture medium for 1 h. After washing, the *Daphnia magna* were further irradiated for 0.5 h under a 660 nm LED lamp and then subjected to the luminescence imaging measurements.

Supplementary Figures

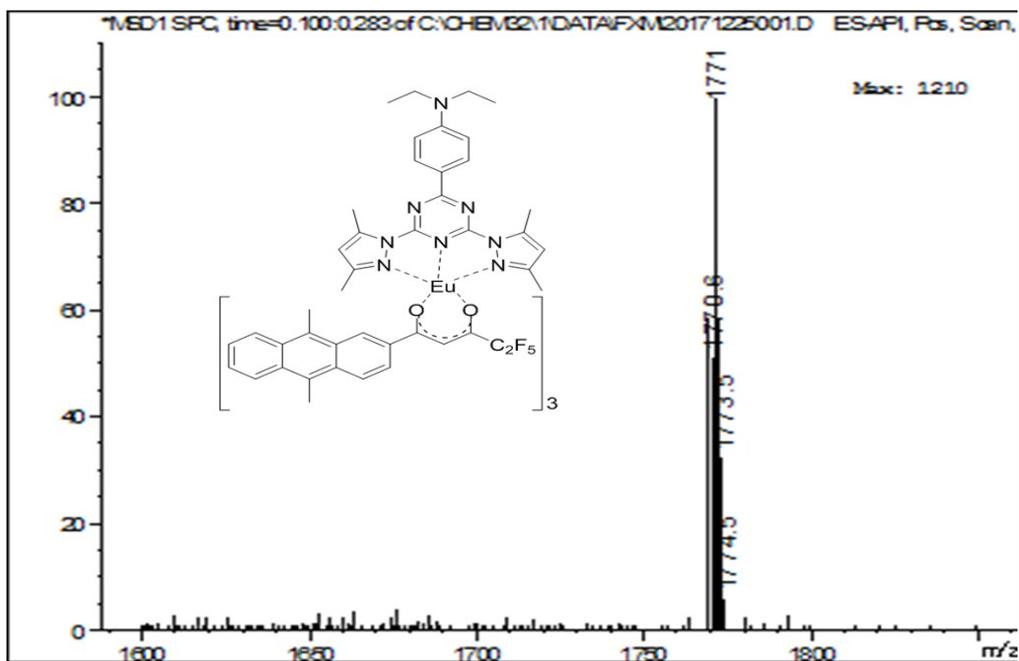


Fig. S1. ESI-MS of $[\text{Eu}(\text{pdap})_3(\text{DPBT})]$.

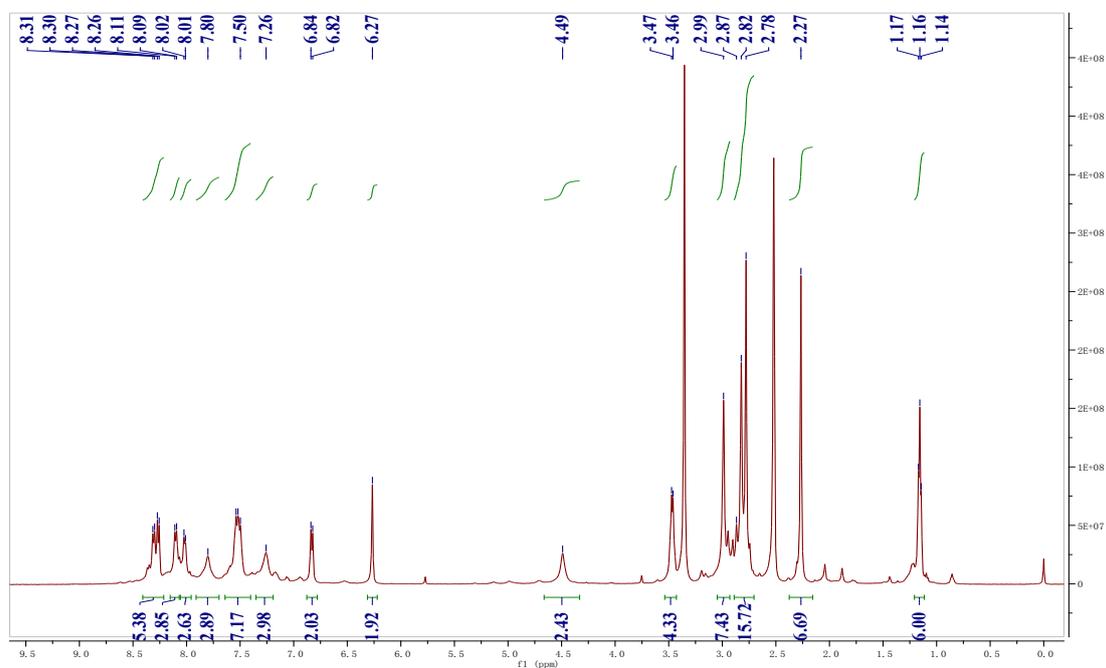


Fig. S2. $^1\text{H-NMR}$ spectrum of $[\text{Eu}(\text{pdap})_3(\text{DPBT})]$.

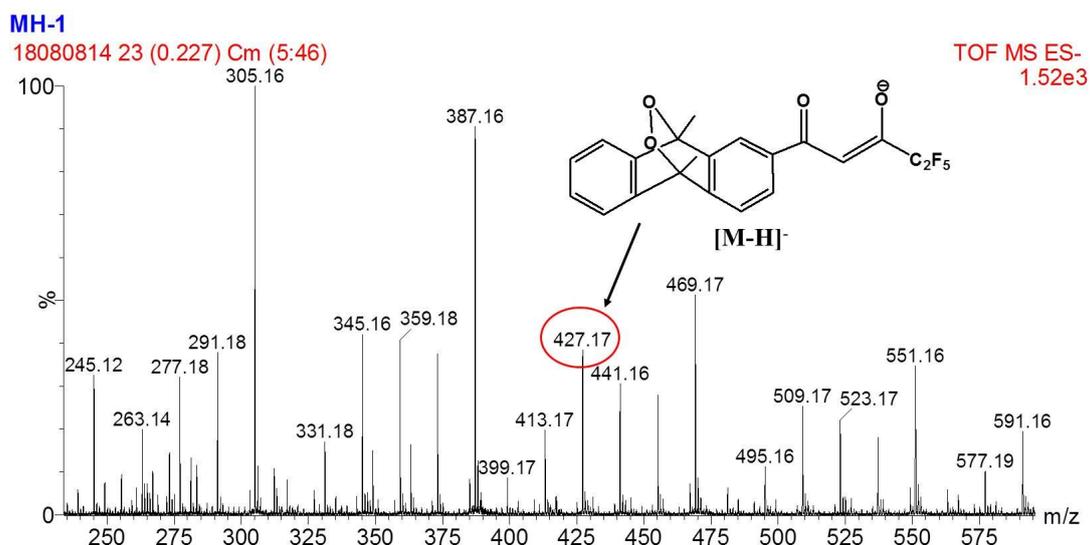


Fig. S3. ESI-MS of the DTPA-treated product of reaction between $[\text{Eu}(\text{pdap})_3(\text{DPBT})]$ and $^1\text{O}_2$.

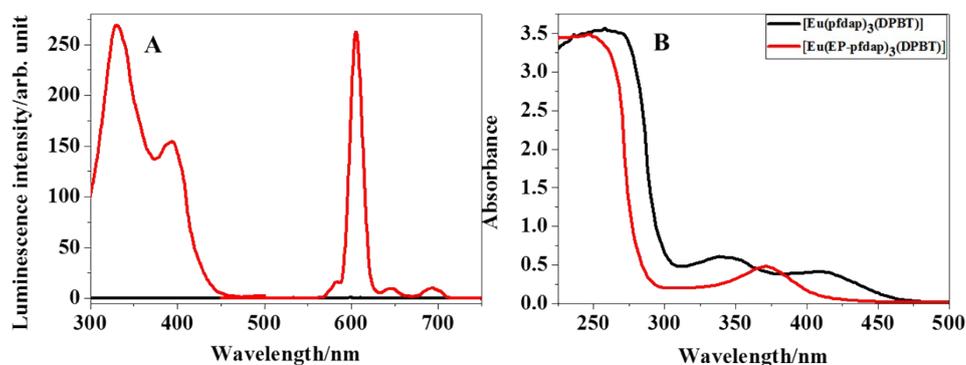


Fig. S4. (A) Time-gated excitation and emission spectra of $[\text{Eu}(\text{pdap})_3(\text{DPBT})]$ (5.0 μM , black) and $[\text{Eu}(\text{EP-pdap})_3(\text{DPBT})]$ (5.0 μM , red) in 0.05 M borate buffer of pH 7.4 containing 0.1% Triton X-100. (B) Absorption spectra of $[\text{Eu}(\text{pdap})_3(\text{DPBT})]$ (5.0 μM , black) and $[\text{Eu}(\text{EP-pdap})_3(\text{DPBT})]$ (5.0 μM , red) in 0.05 M borate buffer of pH 7.4 containing 0.1% Triton X-100.

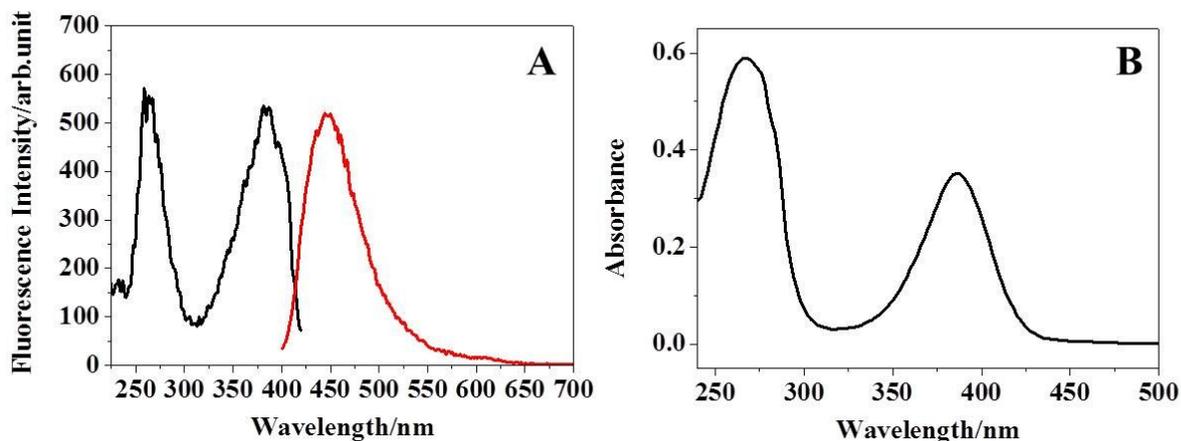


Fig. S5. (A) Steady-state excitation (black) and emission (red) spectra of [Eu(EP-pdap)₃(DPBT)] in 0.05 M borate buffer of pH 7.4 containing 0.1% Triton X-100. (B) Absorption spectrum of DPBT in 0.05 M borate buffer of pH 7.4 containing 0.1% Triton X-100.

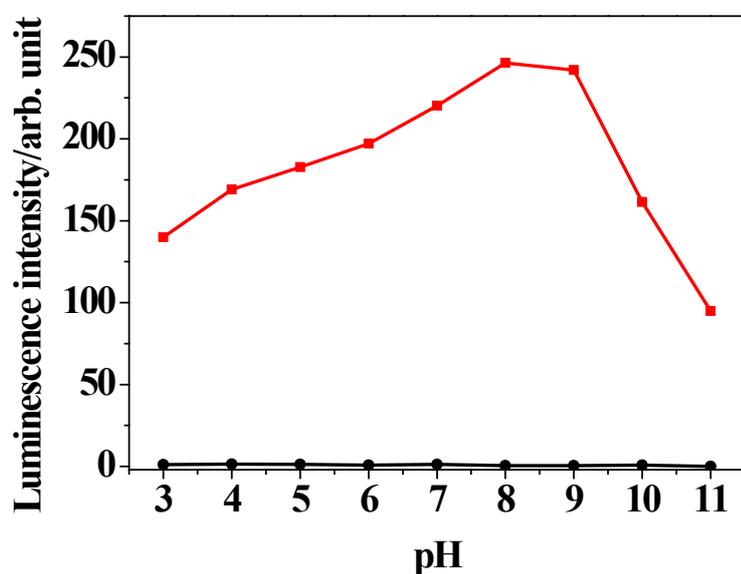


Fig. S6. Effects of pH on the luminescence intensities of [Eu(pdap)₃(DPBT)] (5.0 μM, black line) and [Eu(EP-pdap)₃(DPBT)] (5.0 μM, red line) in 0.05 M borate buffer containing 0.1% Triton X-100.

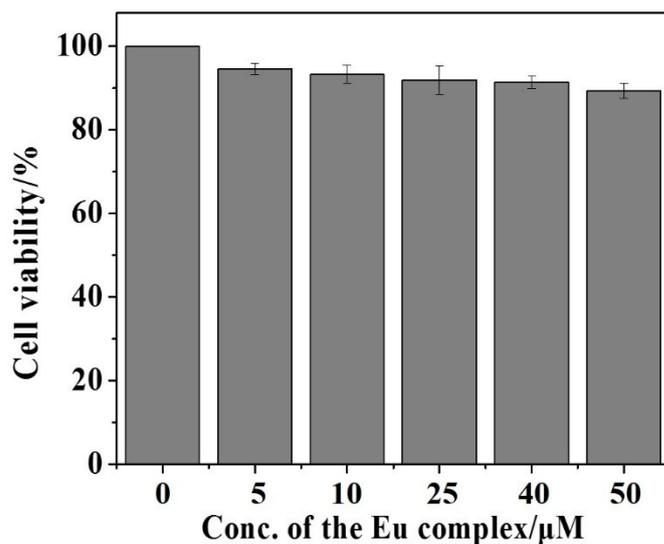


Fig. S7. Viabilities of MCF-7 cells after incubation with different concentrations of $[\text{Eu}(\text{pdap})_3(\text{DPBT})]$ for 24 h.

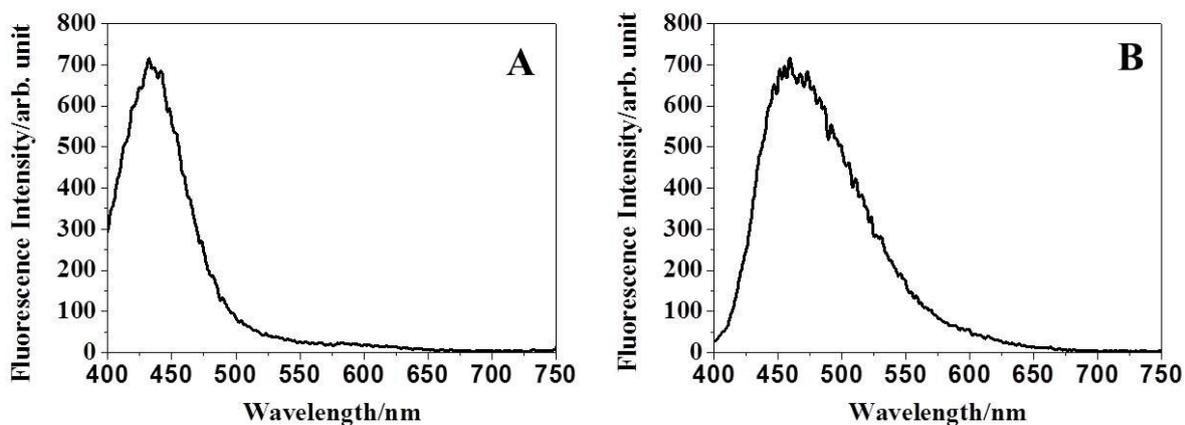


Fig. S8. Steady-state fluorescence spectra of $[\text{Eu}(\text{pdap})_3(\text{DPBT})]$ (A) and DPBT (B) in 0.05 M borate buffer of pH 7.4 containing 0.1% Triton X-100.

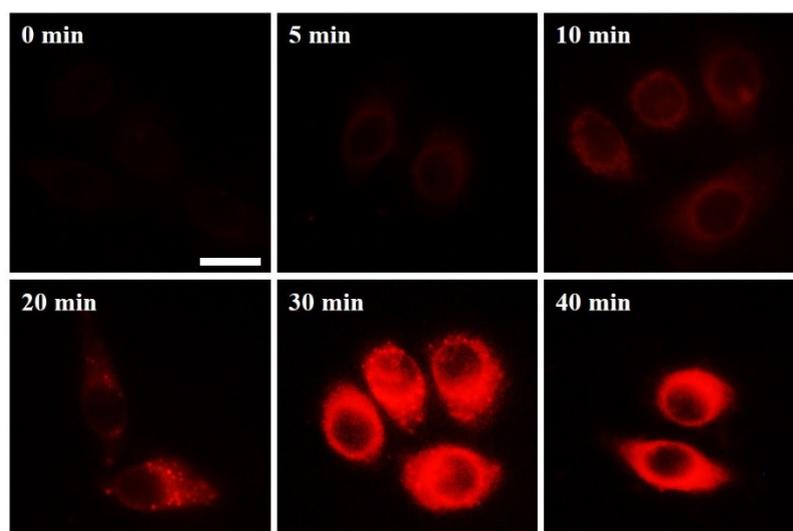


Fig. S9. Time-gated luminescence images of [Eu(pdap)₃(DPBT)]-ALA-loaded MCF-7 cells at different irradiation times. Scale bar: 10 μ m.

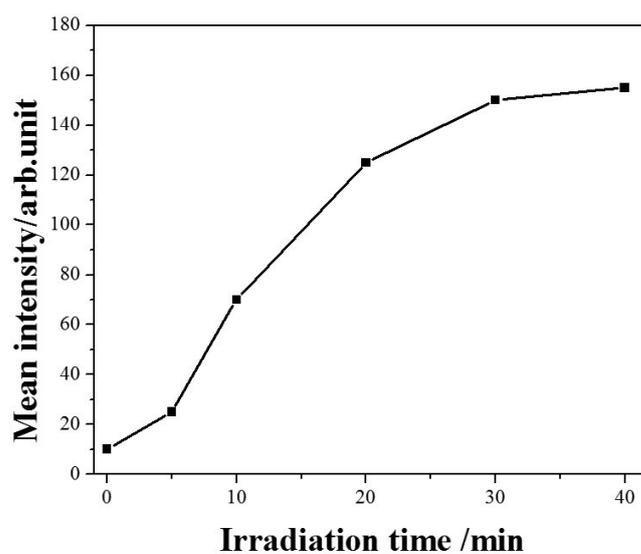


Fig. S10. Intracellular luminescence intensities of the cells in Fig. S9 at different irradiation times.

Reference

1. C. Yang, L. M. Fu, Y. Wang, J. P. Zhang, W. T. Wong, X. C. Ai, Y. F. Qiao, B. S. Zou and L. L. Gui, *Angew. Chem.*, 2004, **116**, 5120–5123.
2. J. Sun, B. Song, Z. Ye and J. Yuan, *Inorg Chem*, 2015, **54**, 11660-11668.
3. L. Zhang, X. Zheng, W. Deng, Y. Lu, S. Lechevallier, Z. Ye, E. M. Goldys, J. M. Dawes, J. A. Piper and J.

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