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Electronic Supplementary Information for

A cell-membrane-permeable europium complex as an efficient luminescent probe for singlet oxygen

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1. Experimental Details

Reagents and Materials. Horseradish peroxidase (HRP, RZ = 3.0, >250 U/mg) was purchased from Bio Basic Inc (Canada). Hematoporphyrin monomethyl ether (HMME) was purchased from Shanghai Xianhui Pharmaceutical Co. Ltd (China). 5-Aminolevulinic acid (ALA) was purchased from Shanghai Fudan-Zhangjiang Bio-Pharmaceutical Co. Ltd (China). 1-Hydroxy-2-oxo-3-(3-amino-propyl)-3-methyl-1-triazene (NOC-13, a NO donor with a half-life of 13.7 min), was synthesized using a reported method.[S1] 3-Morpholinosydnonimine (SIN-1, a $ONOO^-$ donor)[S2] and 3-(4,5-dimethyl-2-thiazoyl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich. Tetrahydrofuran (THF) and acetonitrile were used after appropriate distillation and purification. HeLa cells were obtained from Dalian Medical University. The isotonic saline solution consisting of 140 mM NaCl, 10 mM glucose, and 3.5 mM KCl was prepared in our laboratory. Deionized and distilled water was used throughout. Unless otherwise stated, all chemical materials were purchased from commercial sources and used without further purification.

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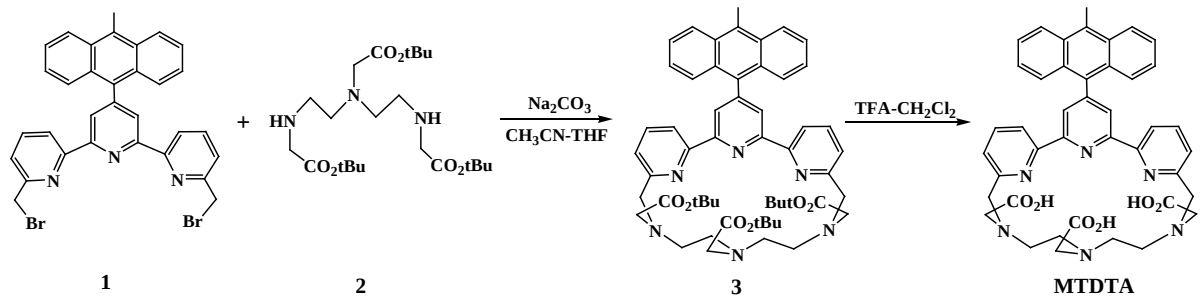
Physical Measurements. NMR spectra were measured on a Bruker Avance spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C). Mass spectra were recorded on a HP1100 LC/MSD MS spectrometer. Elemental analysis was carried out on a Vario-EL analyser. HPLC analysis was carried out on a SinoChrom ODS-BP 5 μm (4.6 × 250 mm) column using an HPLC system composed of two pumps (P230) and a detector (UV 230+). 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, 5 nm. Absorption spectra were measured on a Perkin-Elmer Lambda 35 UV/Vis spectrometer. The time-gated luminescence measurements (Figures 1D, Figure 2B and 2D) were carried out on a Perkin-Elmer Victor 1420 Multilabel Counter with an excitation wavelength of 340 nm, emission wavelength of 615 nm, delay time of 0.2 ms, window time (counting time) of 0.4 ms, and cycling time of 1.0 ms. All bright-field imaging and luminescence imaging were carried out on a laboratory-use luminescence microscope.[S3]

Synthesis of the macrocyclic ligand MTDTA. New macrocyclic ligand MTDTA was synthesized according to the reaction pathway shown in Scheme S1.



Scheme S1. Reaction pathway for the synthesis of MTDTA

(1) Syntheses of compound 1 and 2. Compound **1** and **2** were synthesized according to the literature methods, respectively.[S3,S4] Compound **1** [6,6”-bis(bromomethyl)-4’-(10-methyl-9-anthryl)-2,2’:6’,2”-terpyridine], ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.23 (s, 3H), 4.59 (s, 4H), 7.37-7.41

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(t, *J* = 7.6 Hz, 2H), 7.53-7.57 (m, 4H), 7.71 (d, *J* = 8.8 Hz, 2H), 7.96 (s, 2H), 8.43 (d, *J* = 9.2 Hz, 2H), 8.63 (s, 2H), 8.70 (d, *J* = 7.6 Hz, 2H). Compound **2** [tri-*t*-butyl diethylenetriamine-N^1,N^2,N^3-triacetate], ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.45 (s, 9H), 1.46 (s, 18H), 2.66-2.69 (t, *J* = 5.6 Hz, 4H), 2.80-2.83 (t, *J* = 5.6 Hz, 4H), 2.98 (s, 2H), 3.24 (s, 6H).

(2) Synthesis of compound 3. To a solution of compound **1** (300 mg, 0.49 mmol) in 120 mL of anhydrous CH_3CN and 100 mL of anhydrous THF were added compound **2** (218 mg, 0.49 mmol) and anhydrous Na_2CO_3 (519 mg, 4.9 mmol). After the solution was refluxed for 24 h, the solution was cooled to room temperature and filtered. The filtrate was evaporated, and the residue was purified by silica gel column using ethyl acetate-triethylamine-methanol (40:8:5, v/v/v) as the eluent, and then further purified by alumina column using chloroform-methanol (25:1, v/v) as the eluent. Compound **3** was obtained as a yellowish oil (120 mg, 27.4% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.18 (s, 9H), 1.30 (s, 18H), 2.82 (s, 4H), 2.98 (d, *J* = 8.0 Hz, 4H), 3.23-3.26 (m, 9H), 4.15 (s, 4H), 7.39-7.42 (m, 4H), 7.56-7.63 (m, 4H), 7.85 (d, *J* =4.0 Hz, 4H), 8.08 (s, 2H), 8.46 (d, *J* = 8.0 Hz, 2H). ESI-MS (m/z): 893.5 $[M + H]^-$.

(3) Synthesis of MTDTA. Compound **3** (200 mg, 0.28 mmol) was dissolved in a mixture of 30 mL CH_2Cl_2-CF_3COOH (1:1, v/v) and stirred for 20 h at room temperature. After the solvent was evaporated, the residue was dissolved in 5.0 mL of methanol, and then 50 mL of diethyl ether was added to result in the formation of a precipitate. The precipitate was collected by filtration. After drying, the precipitate was added to 10 mL of dry acetonitrile, and the mixture was refluxed for 20 min. The precipitate was centrifuged and dried to afford MTDTA as a yellowish solid (150 mg, 73.9% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.76 (d, *J* = 8.0 Hz, 4H), 2.91 (d, *J* = 12 Hz, 4H), 3.21-3.45 (m, 9H), 4.02 (s, 4H), 7.45 (d, *J* = 7.6 Hz, 2H), 7.49-7.53 (m, 2H), 7.61-7.65 (m, 4H), 7.91 (t, *J* = 8.0 Hz, 2H), 8.26 (d, *J* = 6.8 Hz, 2H), 8.45 (s, 2H), 8.53 (d, *J* = 8.8 Hz, 2H). ^{13}C NMR

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(100 MHz, DMSO-d_6): δ 14.53, 50.22, 50.41, 54.50, 58.02, 122.62, 125.13, 125.54, 125.62, 125.94, 126.55, 126.84, 129.19, 129.63, 132.09, 138.91, 150.35, 155.77, 156.66, 157.95, 172.60. Elemental analysis calcd (%) for $C_{42}H_{40}N_6O_6 \cdot 3.5H_2O$: C 64.03, H 6.01, N 10.67; found (%): C 63.95, H 5.82, N 10.73. ESI-MS (m/z): 747.5 $[M + Na]^+$. HPLC analysis result (Figure S1A): retention time, 4.6 min, eluent, CH_3OH (80%)/TFA (0.1%) aqueous solution; flow rate, 1.0 mL/min.

Synthesis of [MTDTA-Eu^{III}]: A mixture of MTDTA (30 mg, 0.038 mmol) and $EuCl_3 \cdot 6H_2O$ (14.0 mg, 0.038 mmol) in methanol (8.0 mL) and triethylamine (3.0 mL) was refluxed for 2 h. After the solvent was evaporated, 5.0 mL of diethyl ether was added. The precipitate was isolated with centrifugation and then washed three times with acetone. After drying, the product was added to 5 mL of dry acetonitrile, and the mixture was refluxed for 20 min. After the precipitate was isolated with centrifugation and dried, the solid complex of [MTDTA-Eu^{III}] was obtained (19.3 mg, 58.1% yield). ESI-MS (m/z): 897.4 $[M + Na]^+$. Elemental analysis calcd (%) for $C_{42}H_{37}N_6O_6Eu \cdot C_6H_{15}N \cdot 0.5H_2O$ ([MTDTA-Eu^{III}]·$NEt_3 \cdot 0.5H_2O$): C 58.59, H 5.43, N 9.96; found %): C 58.68, H 5.80, N 10.16. HPLC analysis result (Figure S1B): retention time, 6.9 min, eluent, CH_3OH (80%)/TFA (0.1%) aqueous solution; flow rate, 1.0 mL/min.

Synthesis of [EP-MTDTA-Eu^{III}]: MTDTA (19.7 mg, 0.025 mmol) and $EuCl_3 \cdot 6H_2O$ (9.2 mg, 0.025 mmol) were added to 10 mL of 0.1 M carbonate buffer at pH 10.5. After the solution was stirred for 2 h at room temperature, $Na_2MoO_4 \cdot 2H_2O$ (12.1 mg, 0.05 mmol) and 30% H_2O_2 (200 μL) were added. After the solution was stirred for 30 min, another 30% H_2O_2 (200 μL) was further added to the solution, and then the reaction was monitored by fluorometry to check the complete conversion of [MTDTA-Eu^{III}] to [EP-MTDTA-Eu^{III}]. ESI-MS (m/z): 929.2 $[M + Na]^+$. HPLC analysis result (Figure S1C): retention time, 4.7 min, eluent, CH_3OH (80%)/TFA (0.1%) aqueous solution; flow rate, 1.0 mL/min. The above solution was used as the stock solution of

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[EP-MTDTA-Eu^{III}] for the luminescence property characterizations after suitable dilutions.

Reactions of the probe with various ROS/RNS. All the reactions were carried out in a 0.05 M Tris-HCl buffer at pH 7.4 with the same concentration of the probe (0.1 μM) for 2 h at room temperature. Hydroxyl radical (·OH) was generated in the Fenton system from ferrous ammonium sulfate and hydrogen peroxide.[S5] Nitric oxide (NO) was generated by using NOC-13 as a donor.[S1] Peroxynitrite ($ONOO^-$) was generated by using 3-morpholinosydnonimine (SIN-1) as a donor.[S2] Superoxide anion radical ($O_2^{\cdot-}$) was generated from the xanthine-xanthine oxidase system.[S6] A stock solution of ClO^- was prepared from the commercial sodium hypochlorite solution. Hydrogen peroxide was diluted immediately from a stabilized 30% solution and was assayed by using its molar absorption coefficient of 43.6 $M^{-1}cm^{-1}$ at 240 nm.[S7] Singlet oxygen ($^1O_2$) was chemically generated from the NaOCl-H_2O_2 system.

Detection of $^1O_2$ in Aqueous Media. The probe [MTDTA-Eu^{III}] was used for the detection of $^1O_2$ generated from a Na_2MoO_4-H_2O_2 system, a NaOCl-H_2O_2 system and a HRP-indole-3-acetic acid (IAA) system, respectively. The detailed experimental procedures are as follows:

(1) The reaction of the probe with $^1O_2$ generated from a Na_2MoO_4-H_2O_2 system was performed in 0.1 M carbonate buffer of pH 10.5. Various concentrations of H_2O_2 solutions were added to the buffer solutions containing 10 μM of the probe and 1.0 mM of Na_2MoO_4. After stirring for 4 h at room temperature, the excitation and emission spectra of the solutions were measured with a time-gated mode.

(2) The reaction of the probe with $^1O_2$ generated from a NaOCl-H_2O_2 system was carried out in 0.05 M Tris-HCl buffer of pH 7.4. Various concentrations of NaOCl solutions were added to the buffer solutions containing 10 μM of the probe and 50 mM of H_2O_2. After stirring for 2 h at room temperature, the excitation and emission spectra of the solutions were measured with a time-gated

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mode.

(3) The reaction of the probe with $^1O_2$ generated from a HRP-IAA system was carried out in 0.05 M acetate buffer of pH 4.0. After the IAA solutions with different concentrations were added into the buffer solutions containing 5.0 μM of the probe and 0.5 μM HRP under constant stirring, the real-time emission intensity response of the probe at 608 nm to the addition of IAA was recorded simultaneously on a Perkin-Elmer LS 50B luminescence spectrometer with a time-gated mode.

Luminescence Imaging of the PDT Drug-Induced $^1O_2$ Generation in HeLa Cells. HeLa cells were cultured in RPMI-1640 medium, supplemented with 10% fetal bovine serum, 1% penicillin, and 1% streptomycin at 37 °C in a 5% CO_2/95% air incubator. The concentrated stock solution of [MTDTA-Eu^{III}] (0.5 M) was prepared by dissolving solid [MTDTA-Eu^{III}] in dimethylsulfoxide (DMSO). Before cell loading, the solution was 1000-fold diluted with the isotonic saline solution consisting of 140 mM NaCl, 10 mM glucose and 3.5 mM KCl (final concentration of the probe: 0.5 mM).

(1) Luminescent imaging of the [MTDTA-Eu^{III}]-HMME co-loaded HeLa cells. The cells in a 25 cm^2 glass culture bottle were washed with the isotonic saline solution, and then incubated with the isotonic saline solution containing 0.5 mM of [MTDTA-Eu^{III}] for 3.5 h at 37 °C in the incubator. The cells were washed three times with the isotonic saline solution, and then further incubated with the isotonic saline solution containing 10 μM of HMME for 0.5 h. After washing, the cells incubated in the isotonic saline solution were used for the luminescence imaging measurement on the microscope using the microscopy-equipped 100 W Hg lamp (the light of 450-490 nm) as the irradiation source. For comparison, the [MTDTA-Eu^{III}]-loaded and HMME-loaded HeLa cells were also prepared, respectively, and used for the luminescence imaging measurements.

(2) Luminescent imaging of the [MTDTA-Eu^{III}]-ALA co-loaded HeLa cells. The cells in a 25

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cm^2 glass culture bottle were washed with the isotonic saline solution, and then incubated with the isotonic saline solution containing 0.5 mM of [MTDTA-Eu^{III}] and 4.0 μM of ALA for 3.5 h at 37 °C in the incubator. After washing, the cells incubated in the isotonic saline solution were used for the luminescence imaging measurement on the microscope using the microscopy-equipped 100 W Hg lamp (the light of 450-490 nm) as the irradiation source.

The microscope (TE2000-E; Nikon), equipped with a 100 W mercury lamp, a UV-2A filters (Nikon, excitation filter, 330-380 nm; dichroic mirror, 400 nm; emission filter, >420 nm) and a color CCD camera system (RET-2000R-F-CLR-12-C, Qimaging), was used for the steady-state luminescence imaging measurement with an exposure time of 1 s. The microscope, equipped with a 30 W xenon flash-lamp (Pulse300, Photonic Research Systems), UV-2A filters and a time-gated digital black-and-white CCD camera system (Photonic Research Systems) was used for the time-gated luminescence imaging measurement with the conditions of delay time, 100 μs; gate time, 1000 μs; lamp pulse width, 6 μs; and exposure time, 30 s. The time-gated luminescence images are shown in pseudo-color treated by a SimplePCI software.[S3]

MTT Assay. The *in vitro* cytotoxicity of [MTDTA-Eu^{III}] to HeLa cells was measured by the MTT test using the previously described method.[S8] HeLa cells, cultured in RPMI-1640 medium, supplemented with 10% fetal bovine serum, 1% penicillin and 1% streptomycin, were washed with the isotonic saline solution, and then incubated with the isotonic saline solution containing different concentrations of [MTDTA-Eu^{III}] (0, 0.5 and 1.0 mM) for 4.5 h at 37 °C in a 5% CO_2/95% air incubator. The culture medium was removed, and the cells were further incubated with the isotonic saline solution containing 250 μg/mL of MTT for 5 h in the incubator. After the supernatants were removed, the cells were dissolved in DMSO and then the absorbance at 540 nm was measured.

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2. Supplementary Table

Table S1. Luminescence Properties of [MTDTA-Eu^{III}] and [EP-MTDTA-Eu^{III}][a]

complex	$\lambda_{ex,\ max}$ (nm)	$\lambda_{em,\ max}$ (nm)	ϕ (%)	ε_{335nm} ($cm^{-1}M^{-1}$)	τ (ms)
[MTDTA-Eu^{III}]	288, 335	608	0.88	1.30×10^4	0.31
[EP-MTDTA-Eu^{III}]	288,335	608	13.4	1.01×10^4	0.96

[a]All data were obtained in 0.05 M borate buffer at pH 9.1.

3. Supplementary Figures

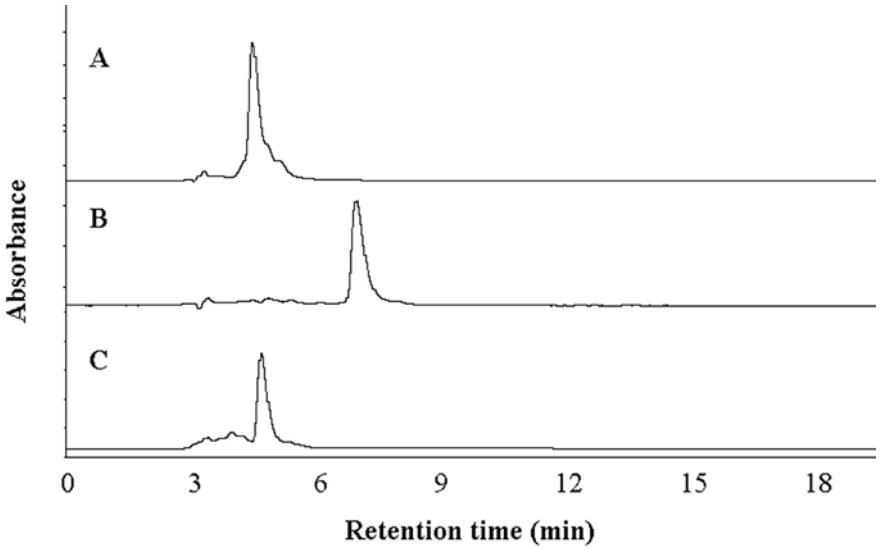

Fig. S1. HPLC chromatograms of MTDTA (A), [MTDTA-Eu^{III}] (B) and [EP-MTDTA-Eu^{III}] (C). The elution was monitored at 330 nm.

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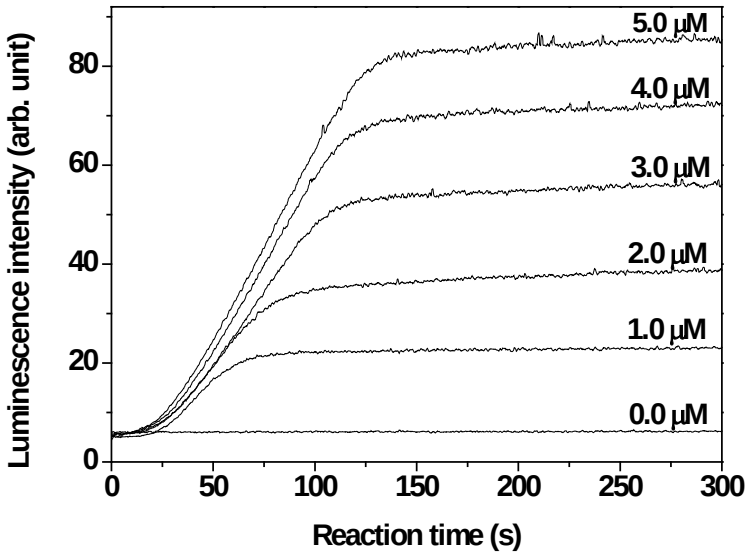

Fig. S2. Temporal dynamics of the luminescence response of [MTDTA-Eu^{III}] (5.0 μM) to $^1O_2$ generated from the IAA-HRP (0.5 μM) system at different IAA concentrations in 0.05 M acetate buffer of pH 4.0.

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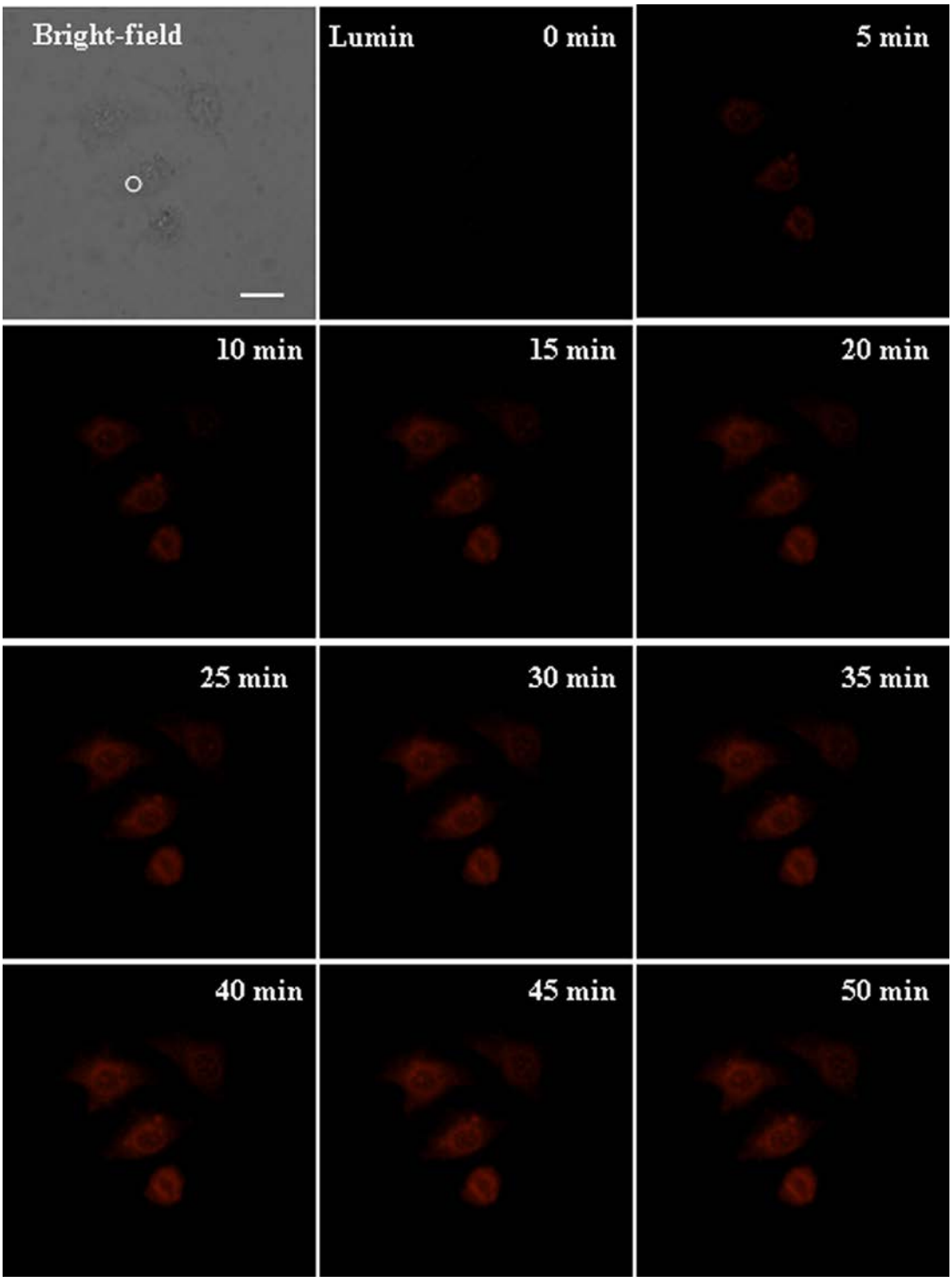

Fig. S3. Bright-field (the circle shows a region near nuclear membrane) and time-gated luminescence images of the HMME-[MTDTA-Eu^{III}] co-loaded HeLa cells at different irradiation times. The time-gated luminescence images are shown in pseudo-color treated by a SimplePCI software.[S3]

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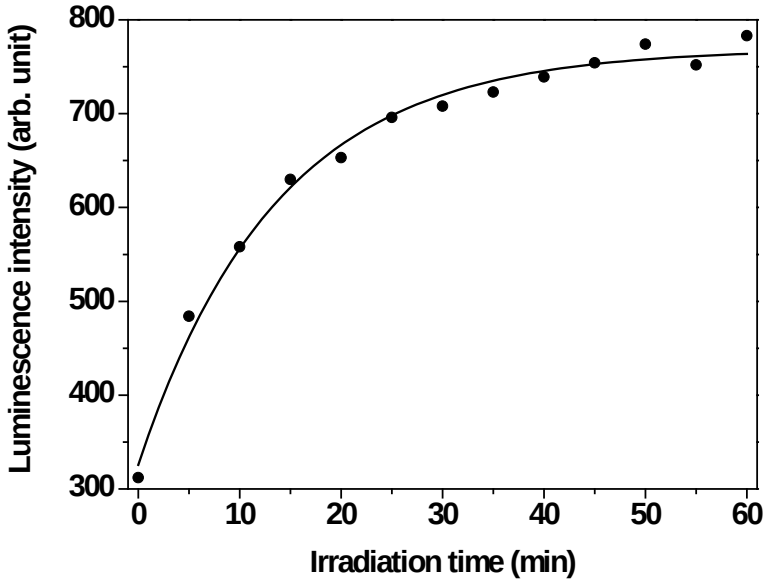

Fig. S4. Luminescence intensities of the circle region in Figure S2 at different irradiation times.

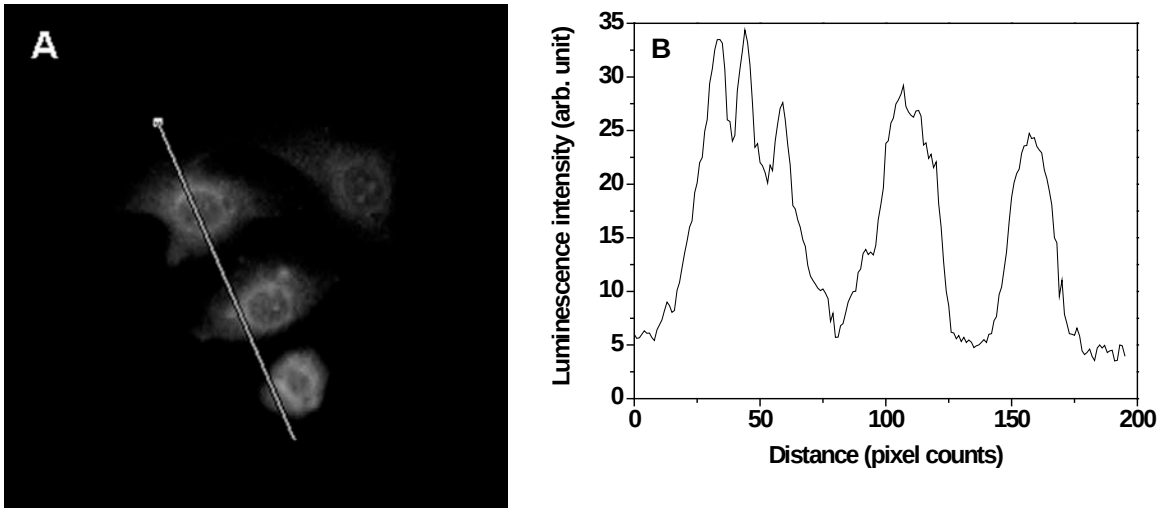

Fig. S5. (A) Time-gated luminescence images (in black-and-white) of the HMME-[MTDTA-Eu^{III}] co-loaded HeLa cells after 50 min irradiation of the 100 W Hg lamp. (B) The luminescence intensity profile along the line in the image.

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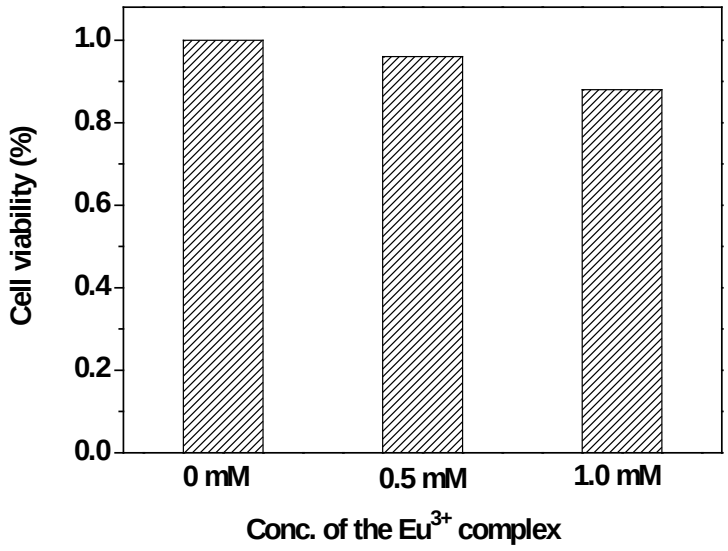

Fig. S6. Viabilities of the HeLa cells after incubated with different concentrations of [MTDTA-Eu^{III}] for 4.5 h.

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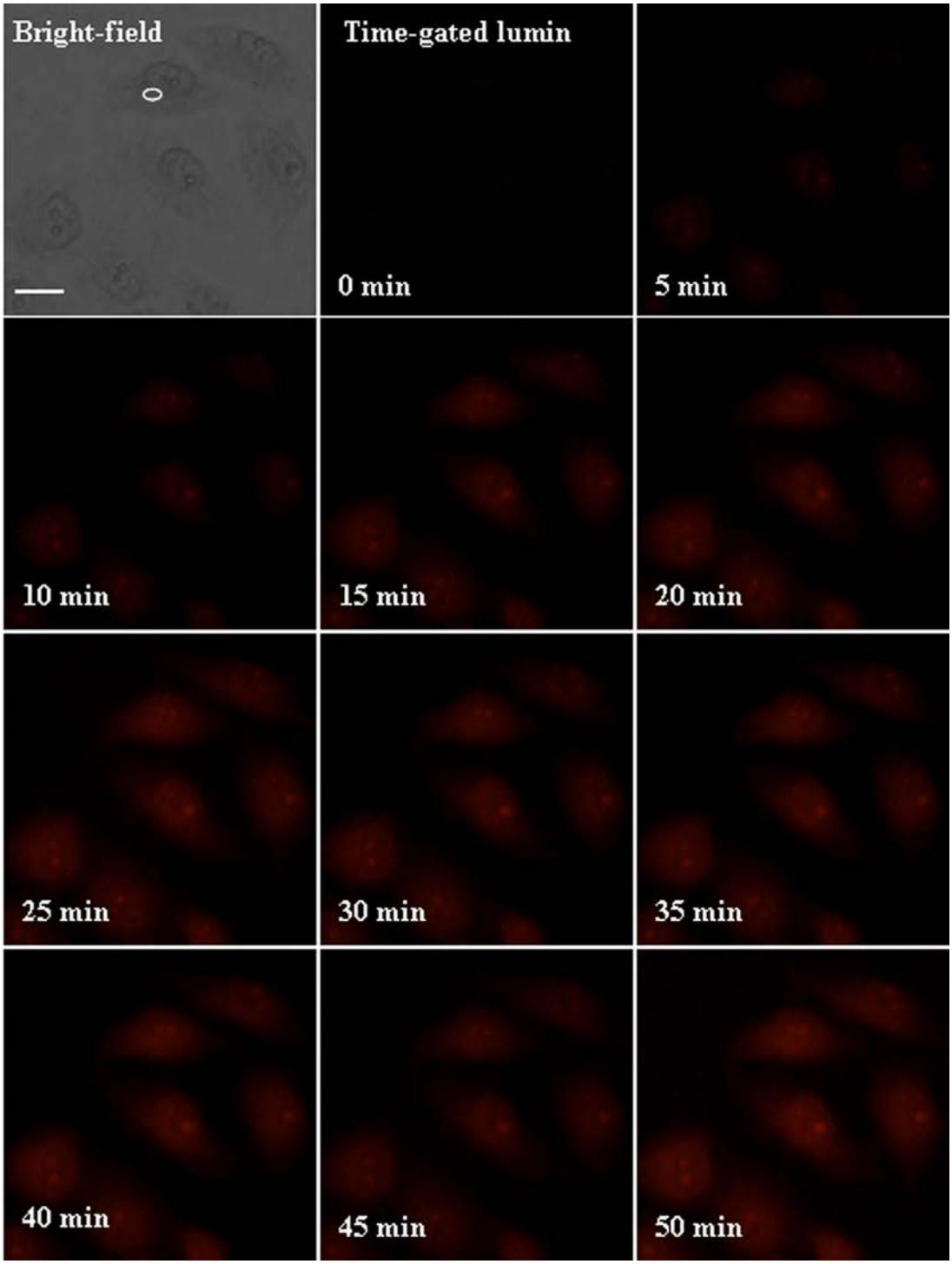

Fig. S7. Bright-field (the ellipse shows a selected region near nuclear membrane) and time-gated luminescence images of the ALA-[MTDTA-Eu^{III}] co-loaded HeLa cells at different irradiation times. The time-gated luminescence images are shown in pseudo-color treated by a SimplePCI software.[S3]

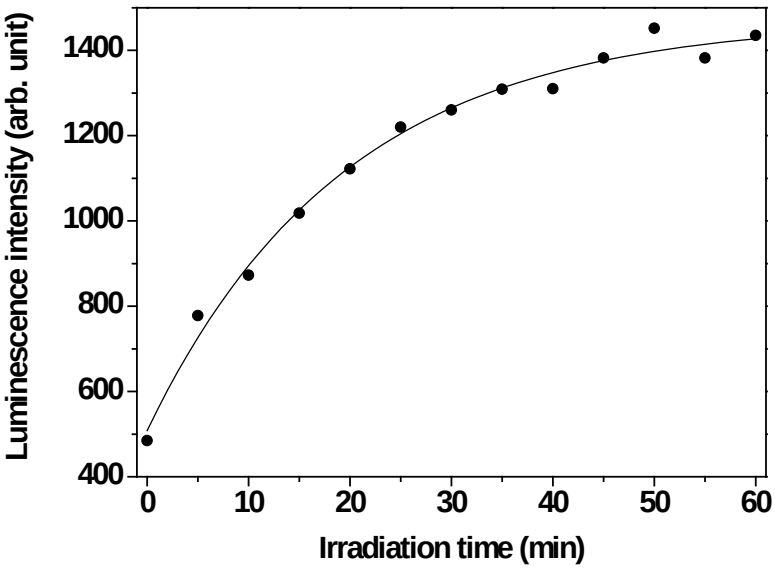

Fig. S8. Luminescence intensities of the ellipse region in Figure S6 at different irradiation times.

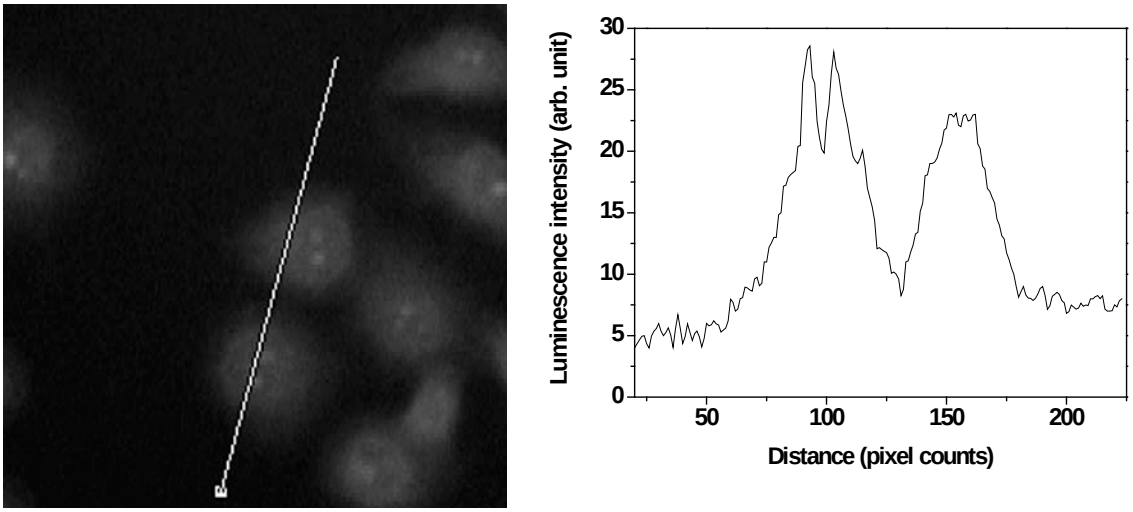

Fig. S9. Time-gated luminescence images (in black-and-white) of the ALA-[MTDTA-Eu^{III}] co-loaded HeLa cells after 50 min irradiation of the Hg lamp (left) and the luminescence intensity profile along the line in the image (right).

References

(S1) J. A. Hrabie, J. R. Klose, D. A. Wink, L. K. Keefer, *J. Org. Chem.*, 1993, **58**, 1472-1476.

(S2) C. Song, Z. Ye, G. Wang, J. Yuan, Y. Guan, *Chem. Eur. J.*, 2010, **16**, 6464-6472.

(S3) B. Song, G. Wang, M. Tan, J. Yuan, *J. Am. Chem. Soc.*, 2006, **128**, 13442-13450.

(S4) C. Galaup, J. M. Couchet, S. Bedel, P. Tisnès, C. Picard, *J. Org. Chem.*, 2005, **70**, 2274-2284.

(S5) K. Setsukinai, Y. Urano, K. Kakinuma, H. J. Majima, T. Nagano, *J.Biol. Chem*., 2003, **278**, 3170-3175.

(S6) K. Xu, X. Liu, B. Tang, G. Yang, Y. Yang, L. An, *Chem. Eur. J.*, 2007, **13**, 1411-1416.

(S7) B. Lei, N. Adachi, T. Arai, *Brain Res. Protoc.*, 1998, **3**, 33-36.

(S8) U. Schatzschneider, J. Niesel, I. Ott, R. Gust, H. Alborzinia, S. Wölfl, *ChemMedChem*, 2008, **3**, 1104-1109.