Electronic Supplementary Information

A europium(III) chelate as an efficient time-gated luminescent probe for nitric oxide

Yonggang Chen, Weihua Guo, Zhiqiang Ye*, Guilan Wang, Jingli Yuan*

*To whom correspondence should be addressed. E-mail: zhiqiangye2001@yahoo.com.cn, jingliyuan@yahoo.com.cn; Phone/fax: +86-411-84706293.

Experimental details

1. Materials and physical measurements

Bromomethyl acetate, anhydrous dimethyl sulfoxide and 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (c-PTIO) were purchased from Sigma-Aldrich. Anhydrous *N,N*-dimethylformamide (DMF) and benzoyl chloride were purchased from Acros. Methanol and acetonitrile were purified and distilled prior to use. Deionized and distilled water was used throughout all the experiments. Fresh *onion* inner-layer epidermal peels were obtained from the Department of Biotechnology, Dalian Institute of Chemical Physics, Chinese Academy of Sciences. Unless otherwise stated, all chemical materials were purchased from commercial sources and used without further purification.

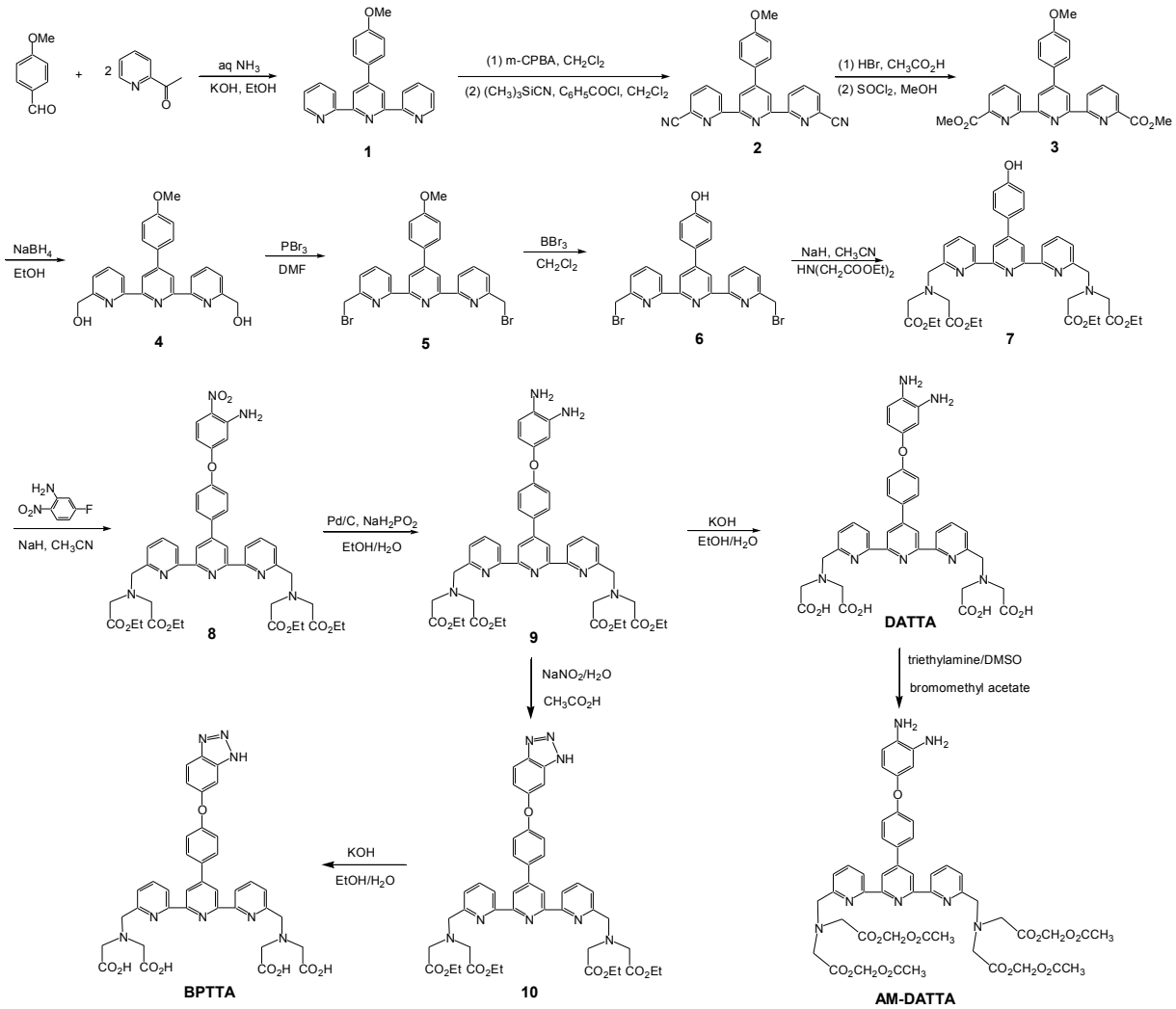
The NMR spectra were recorded on a Bruker Avance 400 spectrometer with tetramethyl silane (TMS) as the internal reference, 400 MHz for ^{1}H and 100 MHz for ^{13}C, respectively. Mass spectra were recorded on an HP 100LC/MSD electrospray ionization (ESI) mass spectrometry, and ESI-HRMS were recorded on a UPCL/Q-TOF MS (Micromass) mass spectrometer. Melting point determinations were performed by using a X-6 digital melting-point apparatus (Beijing Tech Instrument Co. Ltd.). Elemental analysis was performed on a Vanio-EL CHN analyser. Absorption spectra were measured on a Perkin-Elmer Lambda 35 UV-vis spectrometer. Luminescence lifetime was measured on a Perkin-Elmer LS 50B luminescence spectrometer. Time-gated luminescence

spectra were measured on a Perkin-Elmer LS 50B luminescence spectrometer with the conditions of delay time, 0.2 ms; gate time, 0.4 ms; cycle time, 20 ms; excitation slit, 10 nm; and emission slit, 5 nm. The reaction kinetic curves of DATTA-Eu^{3+} with different concentrations of NO (Fig S4) were measured on a Perkin-Elmer LS 50B luminescence spectrometer with time-gated mode under the conditions of delay time, 0.1 ms; gate time, 1.0 ms, cycle time, 20 ms; excitation wavelength, 326 nm; emission wavelength, 612 nm; excitation slit, 10 nm; emission slit, 5 nm; data interval, 1.0 s. The time-gated luminescence measurements for Fig. S3, Fig. S5 and Fig. S6 were carried out on a Perkin-Elmer Victor 1420 multilabel counter with the conditions of excitation wavelength, 340 nm; emission wavelength, 615 nm; delay time, 0.2 ms; window time (counting time), 0.4 ms; and cycling time, 1.0 ms. The luminescence quantum yields were measured with a previous method[S1] by using a Eu^{3+} chelate of (4'-phenyl-2,2':6',2"-terpyridine-6,6"-diyl)bis(methylenenitrilo) tetrakis(acetate) as a reference. The time-gated luminescence imaging measurements were carried out on a laboratory-use luminescence microscope.[S1] The microscope, equipped with a 30 W xenon flash-lamp (Pulse300, Photonic Research Systems Ltd.), UV-2A filters (Nikon, excitation filter, 330-380 nm; dichroic mirror, 400 nm; emission filter, > 420 nm) and a time-gated digital black-and-white CCD camera system (Photonic Research Systems Ltd.), was used for the time-resolved luminescence imaging measurement with the conditions of delay time, 100 μs; gate time, 1000 μs; lamp pulse width, 6 μs; and exposure time, 60 s. The time-gated luminescence image is shown in pseudo-color (wavelength of 615 nm) treated by a SimplePCI software.[S1]

2. Synthesis procedures of the new Eu^{3+} ligands

Scheme S1 shows the synthesis procedures of the new ligands DATTA, BPTTA and AM-DATTA. The details are described as follows.

(i) Synthesis of 4'-(4-methoxyphenyl)-2,2':6',2"-terpyridine (1). To a solution of 2-acetylpyridine (24.2 g, 0.2 mol) in ethanol (300 mL) was added aqueous KOH (10 M, 20 mL), and stirred at room temperature for 30 min. To the solution was added 4-methoxybenzaldehyde (13.6 g, 0.1 mol) and stirred at room temperature for another 15 min, and then 25% ammonia solution (150 mL) was added. After the reaction mixture was stirred at 50 ºC over night, the suspension was filtered and the precipitate was washed with 50 mL of water and 30 mL of cold methanol. The crude product was recrystallized from EtOH to yield compound **1** (17.0 g, 50% yield); m.p. 167-169 ºC; ^{1}H NMR ($CDCl_3$): δ 8.74 (s, 2H), 8.69 (d, J = 8.0 Hz, 2H), 7.92-7.90 (m, 4H), 7.37 (t, J = 6.4 Hz, 2H), 7.04 (d, J = 8.0 Hz, 2H), 3.89 (s, 3H); ESI-HRMS (*m/z*): calcd for $C_{22}H_{18}N_3O$ 340.1450, found 340.1454, $[M+H]^+$.



Scheme S1. Synthesis procedure of the ligands DATTA, BPTTA and AM-DATTA.

(ii) Synthesis of 4'-(4-methoxyphenyl)-2,2':6',2"-terpyridine-6,6"-dicarbonitrile (2). To a solution of compound **1** (1.82 g, 5.4 mmol) in CH_2Cl_2 (100 mL) was added 3-chloroperbenzoic acid (3.70 g, 21.4 mmol). The solution was stirred at room temperature for 24 h, and washed with 10% Na_2CO_3 solution (2 × 100 mL). The organic phase was dried with anhydrous Na_2SO_4 and evaporated. The residue and trimethylsilylcyanide (3.73 g, 37.5 mmol) were dissolved in CH_2Cl_2 (40 mL). After stirring for 20 min, benzoyl chloride (3.02 g, 21.5 mmol) was added dropwise, and the mixture was stirred at room temperature for 24 h. The mixture was concentrated to half volume, and the aqueous solution of 10% K_2CO_3 (30 mL) was added. The mixture was stirred for 1 h, and the precipitate was collected by filtration and washed with water and acetonitrile. Compound **2** was obtained after recrystallization from dioxane (1.40 g, 67% yield); m.p. 197-199 ºC; ^{1}H NMR ($CDCl_3$): δ 8.86 (d, J = 8.4 Hz, 2H), 8.79 (s, 2H), 8.02 (t, J = 8.0 Hz, 2H), 7.89 (d, J = 8.4 Hz, 2H), 7.76 (d, J = 7.6 Hz, 2H), 7.10 (d, J = 8.4 Hz, 2H), 3.92 (s, 3H); ESI-HRMS (*m/z*): calcd for $C_{24}H_{15}N_5ONa$ 412.1174, found 412.1163, $[M+Na]^+$.

(iii) Synthesis of dimethyl 4'-(4-methoxyphenyl)-2,2':6',2"-terpyridine-6,6"-dicarboxylate (3). The compound **2** (1.56 g, 4.0 mmol) in a mixture of 40% HBr (50 mL) and glacial acetic acid (25 mL) was heated at 110 ºC for 8 h, and then the suspension was poured into 200 mL of ice-water. The precipitate was filtered, washed with water and dried in vacuum. To 120 mL of cooled methanol was added dropwise $SOCl_2$ (4.77 g, 40.1 mmol), and stirred for 20 min at room temperature. The above precipitate was added, and the mixture was refluxed for 18 h. After evaporation, the residue was washed with saturated $NaHCO_3$ solution and dried. The resulting solid was recrystallized from dioxane to yield compound **3** (1.17 g, 64% yield); m.p. 236-239 ºC; ^{1}H NMR ($CDCl_3$): δ 8.84 (d, J = 8.0 Hz, 2H), 8.79 (s, 2H), 8.19 (d, J = 7.6 Hz, 2H), 8.03 (t, J = 8.0 Hz, 2H), 7.88 (d, J = 8.8 Hz, 2H), 7.06 (d, J = 8.4 Hz, 2H), 4.07 (s, 6H), 3.90 (s, 3H); ESI-HRMS (*m/z*): calcd for $C_{26}H_{22}N_3O_5$ 456.1559, found 456.1566, $[M+H]^+$.

(iv) Synthesis of 4'-(4-methoxyphenyl)-2,2':6',2"-terpyridine-6,6"-dimethanol (4). To a suspension of compound **3** (1.50 g, 3.3 mmol) in EtOH (60 mL) was added $NaBH_4$ (0.50 g, 13.2 mmol), and then the mixture was stirred at room temperature for 2 h, and further refluxed for 8 h. After evaporation, 10 mL of saturated $NaHCO_3$ solution was added, and the mixture was heated to boiling for 10 min. The suspension was poured into 30 mL of ice-water, and the precipitate was filtered and washed with water and acetonitrile. Compound **4** was obtained (1.22 g, 93%); m.p. 174-176 ºC; ^{1}H NMR ($CDCl_3$): δ 8.68 (s, 2H), 8.58 (d, J = 7.6 Hz, 2H), 7.89 (t, J = 8.0 Hz, 2H), 7.82 (d, J = 8.4 Hz, 2H), 7.29 (d, J = 7.6 Hz, 2H), 7.08 (d, J = 8.8 Hz, 2H), 4.88 (s, 4H), 4.06 (br, 2H), 3.91 (s, 3H); ESI-HRMS (*m/z*): calcd for $C_{24}H_{22}N_3O_3$ 400.1661, found 400.1658, $[M+H]^+$.

(v) Synthesis of 6,6"-bis(bromomethyl)-4'-(4-methoxyphenyl)-2,2':6',2"-terpyridine (5). After a mixture of dry DMF (70 mL) and PBr_3 (3.65 g, 13.5 mmol) was stirred at room temperature for 15 min, compound **4** (2.17 g, 5.4 mmol) was added, and the mixture was stirred for 24 h at room temperature. The solution was neutralized with saturated $NaHCO_3$ solution, and the precipitate was filtered and washed with water and acetonitrile. Compound **5** was obtained (2.67 g, 94%); m.p. 178 ºC, decomp.; ^{1}H NMR ($CDCl_3$): δ 8.73 (s, 2H), 8.57 (d, J = 7.6 Hz, 2H), 7.86-7.92 (m, 4H), 7.51 (d, J = 7.6 Hz, 2H), 7.08 (d, J = 8.8 Hz, 2H), 4.69 (s, 4H), 3.91 (s, 3H); ESI-HRMS (*m/z*): calcd for $C_{24}H_{20}N_3OBr_2$ 523.9973, found 523.9974, $[M+H]^+$.

(vi) Synthesis of 6,6"-bis(bromomethyl)-4'-(4-hydroxyphenyl)-2,2':6',2"-terpyridine (6). To a solution of compound **5** (1.48 g, 2.8 mmol) in CH_2Cl_2 (130 mL) was added dropwise a solution of BBr_3 (2.82 g, 11.3 mmol) in CH_2Cl_2 (15 mL) at 0 ºC under an argon atmosphere. The mixture was allowed to warm gradually to room temperature and stirred overnight. To the solution was added dropwise 80 mL of cooled water. The mixture was stirred for 30 min and the precipitate was collected by filtration and washed with water. The crude product was recrystallized from methanol to yield compound **6** (0.71 g, 49% yield); m.p. 247 ºC, decomp.; ^{1}H NMR (DMSO-d_6): δ 8.67 (s, 2H), 8.60 (d, J = 7.6 Hz, 2H), 8.08 (t, J = 8.0 Hz, 2H), 7.81 (d, J = 8.4 Hz, 2H), 7.71 (d, J = 7.6 Hz,

2H), 7.02 (d, J = 8.4 Hz, 2H), 4.87 (s, 4H); ESI-HRMS (*m/z*): calcd for $C_{23}H_{18}N_3OBr_2$ 509.9817, found 509.9815, $[M+H]^+$.

(vii) Synthesis of tetraethyl [4'-(4-hydroxyphenyl)-2,2':6',2"-terpyridine-6,6"-diyl] bis(methylene nitrilo) tetrakis(acetate) (7). To a solution of diethyl iminodiacetate (1.02 g, 5.4 mmol) in dry acetonitrile (60 mL) was added NaH (0.13 g, 5.4 mmol) under an argon atmosphere, and the mixture was stirred for 15 min at room temperature. After compound **6** (1.15 g, 2.2 mmol) was added, the mixture was stirred overnight at room temperature, and then filtered. The filtrate was evaporated, and the residue was dissolved in $CHCl_3$ (20 mL). The $CHCl_3$ solution was washed with water (2 × 20 mL), and dried with anhydrous Na_2SO_4. The solvent was removed by evaporation and the residue was subjected to silica gel chromatography eluted with petroleum ether/ethyl acetate (1:1, v/v) to afford a sticky product which was precipitated from ethanol/water (1:5, v/v) solution to yield compound **7** as a white solid (0.67 g, 41% yield); m.p. 113-115 ºC; ^{1}H NMR ($CDCl_3$): δ 8.61 (s, 2H), 8.53 (d, J = 7.6 Hz, 2H), 7.88 (t, J = 7.6 Hz, 2H), 7.69 (d, J = 8.4 Hz, 2H), 7.66 (d, J = 8.0 Hz, 2H), 7.97 (d, J = 8.4 Hz, 2H), 4.22 (s, 4H), 4.16 (q, J = 7.2 Hz, 8H), 3.70 (s, 8H), 1.23 (t, J = 7.2 Hz, 12H); ESI-HRMS (*m/z*): calcd for $C_{39}H_{46}N_5O_9$ 728.3296, found 728.3284, $[M+H]^+$.

(viii) Synthesis of tetraethyl {4'-[4-(3-amino-4-nitrophenoxy)phenyl]-2,2':6',2"-terpyridine-6,6"-diyl}bis(methylenenitrilo) tetrakis(acetate) (8). To a solution of compound **7** (0.73 g, 1.0 mmol) in dry acetonitrile (35 mL) was added NaH (0.024 g, 1.0 mmol) under an argon atmosphere, and the solution was stirred for 15 min at room temperature. After 5-fluoro-2-nitroaniline (0.23 g, 1.5 mmol) was added, the mixture was stirred at 50 ºC for 8 h. The solution was filtered, and the filtrate was evaporated to give a residue which was dissolved in $CHCl_3$ (20 mL), and washed with water (2 × 20 mL), and dried with anhydrous Na_2SO_4. After evaporation, the crude product was purified by silica gel chromatography eluted with petroleum ether/ethyl acetate (2:1, v/v) to yield compound **8** as bright yellow oil (0.71 g, 82% yield); ^{1}H NMR ($CDCl_3$): δ 8.71 (s, 2H), 8.56 (d, J = 7.6 Hz, 2H), 8.13 (d, J = 9.6 Hz, 1H), 7.92 (d, J = 8.4 Hz, 2H), 7.88 (t, J = 8.0 Hz, 2H), 7.65 (d, J = 7.6 Hz, 2H), 7.22 (d, J = 8.0 Hz, 2H), 6.39 (d, J = 9.6 Hz, 1H), 6.35 (br, 2H), 6.27 (s, 1H), 4.21 (s, 4H), 4.16 (q, J = 7.2 Hz, 8H), 3.70 (s, 8H), 1.24 (t, J = 7.2 Hz, 12H); ESI-HRMS (*m/z*): calcd for $C_{45}H_{50}N_7O_{11}$ 864.3568, found 864.3559, $[M+H]^+$.

(ix) Synthesis of tetraethyl {4'-[4-(3,4-diaminophenoxy)phenyl]-2,2':6',2"-terpyridine-6,6"-diyl}bis(methylenenitrilo) tetrakis(acetate) (9). To a mixture of compound **8** (0.82 g, 0.95 mmol) and 10% Pd/C (0.05 g) in ethanol (40 mL) was added dropwise a solution of NaH_2PO_2 (1.0 g, 11.4 mmol) in water (20 mL) at 50 ºC under an argon atmosphere. After the mixture was refluxed for 6 h, the catalyst was removed by filtration and the solvent was evaporated. The residue was dissolved in $CHCl_3$ (20 mL), and the $CHCl_3$ solution was washed with water (2 × 20 mL) and dried with anhydrous Na_2SO_4. After evaporation, the crude product was purified by silica gel chromatography

eluted with petroleum ether/ethyl acetate (1:5, v/v) to yield compound **9** as colorless oil (0.46 g, 58% yield); ^{1}H NMR ($CDCl_3$): δ 8.68 (s, 2H), 8.54 (d, J = 8.0 Hz, 2H), 7.86 (t, J = 7.6 Hz, 2H), 7.83 (d, J = 8.8 Hz, 2H), 7.63 (d, J = 7.6 Hz, 2H), 7.09 (d, J = 8.8 Hz, 2H), 6.72 (d, J = 8.0 Hz, 1H), 6.50 (s, 1H), 6.46 (d, J = 8.0 Hz, 1H), 4.19 (s, 4H), 4.16 (q, J = 7.2 Hz, 8H), 3.70 (s, 8H), 1.23 (t, J = 7.2 Hz, 12H); ESI-HRMS (*m/z*): calcd for $C_{45}H_{52}N_7O_9$ 834.3827, found 834.3824, $[M+H]^+$.

(x) Synthesis of {4'-[4-(3,4-diaminophenoxy)phenyl]-2,2':6',2"-terpyridine-6,6"-diyl} bis(methylenenitrilo) tetrakis(acetic acid) (DATTA). A mixture of compound **9** (0.46 g, 0.55 mmol), ethanol (20 mL), KOH (0.93 g, 16.6 mmol) and water (5 mL) was stirred at room temperature for 24 h under an argon atmosphere. After evaporation, the residue was dissolved in water (15 mL). To the solution was added dropwise 1 M HCl to adjust the pH to 2-3, and then the suspension was stirred for 3 h at room temperature. The precipitate was filtered and washed with water. The dried solid was refluxed in dry acetonitrile (20 mL) for 10 min. The product was collected by filtration and then dried to yield **DATTA** (0.24 g, 60% yield); m.p. 239 ºC, decomp.; ^{1}H NMR (DMSO-d_6): δ 8.65 (s, 2H), 8.53 (d, J = 7.6 Hz, 2H), 8.01 (t, J = 7.6 Hz, 2H), 7.89 (d, J = 8.4 Hz, 2H), 7.62 (d, J = 7.6 Hz, 2H), 7.08 (d, J = 8.4 Hz, 2H), 6.59 (d, J = 8.4 Hz, 1H), 6.35 (s, 1H), 6.22 (d, J = 8.4 Hz, 1H), 4.10 (s, 4H), 3.57 (s, 8H). ^{13}C NMR (DMSO-d_6): δ 172.93, 160.6, 159.05, 156.0, 154.80, 149.25, 147.72, 138.42, 137.53, 131.17, 128.79, 123.89, 119.83, 117.85, 115.99, 108.74, 106.89, 59.54, 54.91; elemental analysis calcd. (%) for $C_{37}H_{35}N_7O_9 \cdot 1.5H_2O$: C 59.35, H 5.12, N 13.09; found (%): C 58.96, H 5.08, N 12.94; ESI-HRMS (*m/z*): calcd for $C_{37}H_{36}N_7O_9$ 722.1675, found 722.1613, $[M+H]^+$.

(xi) Synthesis of tetraethyl {4'-[4-(benzotriazol-6-yl-oxy)phenyl]-2,2':6',2"-terpyridine-6,6"-diyl}bis(methylenenitrilo) tetrakis(acetate) (10). To a mixture of compound **9** (430 mg, 0.52 mmol), acetic acid (63 mg, 1.04 mmol) and water (4 mL) was added dropwise a solution of $NaNO_2$ (43 mg, 0.62 mmol) in water (2 mL) at 5 ºC. After stirring for 10 min, the mixture was allowed to warm gradually to room temperature for 5 h. To the solution was add $CHCl_3$ (20 mL), and the organic phase was washed with water (2 × 20 mL) and then dried with anhydrous Na_2SO_4. After evaporation, the residue was purified by silica gel chromatography eluted with petroleum ether/ethyl acetate (1:2, v/v) to yield compound **10** as colorless oil (171 mg, 39% yield); ^{1}H NMR ($CDCl_3$): δ 8.64 (s, 2H), 8.54 (d, J = 7.6 Hz, 2H), 7.93 (d, J = 8.8 Hz, 1H), 7.85 (t, J = 7.6 Hz, 2H), 7.79 (d, J = 8.4 Hz, 2H), 7.60 (d, J = 7.6 Hz, 2H), 7.34 (s, 1H), 7.17 (d, J = 7.6 Hz, 1H), 7.12 (d, J = 8.4 Hz, 2H), 4.22 (s, 4H), 4.15 (q, J = 7.2 Hz, 8H), 3.71 (s, 8H), 1.20 (t, J = 7.2 Hz, 12H).

(xii) Synthesis of {4'-[4-(benzotriazol-6-yl-oxy)phenyl]-2,2':6',2"-terpyridine-6,6"-diyl} bis(methylenenitrilo) tetrakis(acetic acid) (BPTTA). A mixture of compound **10** (171 mg, 0.20 mmol), ethanol (20 mL), KOH (336 mg, 6.0 mmol) and water (5 mL) was stirred at room temperature for 24 h. After evaporation, the residue was dissolved in water (15 mL). The solution was added dropwise 1 M HCl to adjust the pH to 2-3, and the suspension was stirred for 3 h at room

temperature. The precipitate was filtered and washed with water. After drying, the solid was refluxed in dry acetonitrile (10 mL) for 10 min. The product was filtered and dried to yield **BPTTA** (56 mg, 38% yield); m.p. 208-211 ºC; ^{1}H NMR (DMSO-d_6): δ 8.71 (s, 2H), 8.58 (d, J = 7.6 Hz, 2H), 8.07-8.00 (m, 5H), 7.66 (d, J = 7.6 Hz, 2H), 7.54 (s, 1H), 7.28-7.26 (m, 3H), 4.20 (s, 4H), 3.68 (s, 8H); ^{13}C NMR (DMSO-d_6): δ 172.55, 158.75, 158.23, 156.02, 154.66, 149.10, 138.63, 133.02, 129.29, 128.67, 124.13, 120.05, 119.54, 118.14, 59.49, 54.90; elemental analysis calcd. (%) for $C_{37}H_{32}N_8O_9 \cdot 4H_2O$: C 55.20, H 5.01, N 13.92; found (%): C 55.41, H 4.88, N 14.12; ESI-HRMS (*m/z*): calcd for $C_{37}H_{33}N_8O_9$ 733.2370, found 733.2357, $[M+H]^+$.

(xiii) Synthesis of acetoxymethyl ester of DATTA (AM-DATTA). To a solution of DATTA·1.5H_2O (11.58 mg, 15.5 μmol) and bromomethyl acetate (61 μL, 618 μmol) in dry DMSO (193 μL) was added dry triethylamine (89 μL, 617 μmol) under an argon atmosphere. After stirring at room temperature overnight, the precipitate was removed by centrifugation. The supernatant was carefully collected and used as the stock solution of **AM-DATTA** without further purification. ESI-MS (m/z): 1009.8, $[M+H]^+$.

3. Reactions of DATTA-Eu^{3+} with different ROS and RNS

All the experiments were carried out in 0.05 M borate buffer of pH 7.4 at room temperature. The solution of DATTA-Eu^{3+} was prepared by in -situ mixing equiv molar of DATTA and $EuCl_3$ in the buffer. The luminescence intensities were recorded after reacting DATTA-Eu^{3+} (0.1 μM) with different ROS (10.0 μM) or RNS (10.0 μM) for 60 min. The solutions (100 μL) were added to the wells of a 96-well microtiter plate, and then subjected to the time-gated luminescence measurement on a Perkin Elmer Victor 1420 multilabel counter.

The NO aqueous solution was prepared by passing NO gas through a 0.05 M argon deoxidized borate buffer of pH 7.4 for 3 h. The NO concentration was measured by using the Griess method.[S2] Peroxynitrite ($ONOO^-$) was synthesized from sodium nitrite (0.6 M) and H_2O_2 (0.65 M) in a quenched-flow reactor (excess H_2O_2 was used to minimize nitrite contamination). After the reaction, the solution was treated with MnO_2 to eliminate the excess H_2O_2. The concentration of the $ONOO^-$ stock solution was determined by measuring the absorbance at 302 nm with a molar extinction coefficient of 1670 $M^{-1}cm^{-1}$.[S3] Hydrogen peroxide (H_2O_2) 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.[S4] Hydroxyl radical (·OH) was generated in the Fenton system from ferrous ammonium sulfate and hydrogen peroxide.[S5] Singlet oxygen ($^1O_2$) was chemically generated by the reaction of hypochlorite with hydrogen peroxide.[S6] Superoxide solution ($O_2^{-\cdot}$) was generated from a mixture of xanthine (0.1 mM) and xanthine oxidase (0.1 unit/mL).[S7] The freshly prepared aqueous solutions of

NaOCl, $NaNO_2$ and $NaNO_3$ were used as hypochlorite anion (^-OCl), nitrite (NO_2^-) and nitrate (NO_3^-) sources, respectively.

4. Luminescence imaging of NO in plant tissues

The luminescence imaging detection of the NO production in living plant tissues was carried out with an experimental procedure similar to the reported method.[S8] After fresh *onion* inner-layer epidermal peels were incubated in the dark for 60 min at 25 ºC in 0.05 M Tris-HCl buffer of pH 7.4 containing AM-DATTA (200 μM) and Eu^{3+} (200 μM), the peels were washed for 10 min with 0.05 M Tris-HCl buffer of pH 7.4, and then subjected to the luminescence microscopy imaging measurement. To verify that the luminescence signals were attributed to the NO evolution, a control experiment was carried out by adding a NO scavenger,[S8] 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (c-PTIO, 200 μM), into the incubation solution.

5. Quantitative luminescence detection of the intercellular NO concentration in the incubation solution of the plant tissue samples

Since NO can diffuse rapidly across biological membranes to travel for hundreds of microns in biological systems,[S9] to evaluate the performance of DATTA-Eu^{3+} for the quantitative luminescence detection of NO in plant tissue samples, the intercellular NO concentration in the incubation solution of the *onion* inner-layer epidermal peels was measured by using the cell membrane-impermeable DATTA-Eu^{3+} as a probe. After 50 mg of fresh *onion* inner-layer epidermal peels were incubated in the dark for 60 min at 25 ºC in 2.0 mL of 0.05 M borate buffer of pH 7.4 containing 0.1 μM DATTA-Eu^{3+}, the peels were removed, and the time-gated luminescence intensity of the incubation solution was measured (Fig. S6). Based on the calibration curve measured under the same conditions (Fig. S3), the NO concentration in the solution was calibrated to be ~50 nM.

Supplementary Table and Figures

Table S1. Luminescence properties of DATTA-Eu^{3+} and BPTTA-Eu^{3+*}

chelate	$\lambda_{ex,max}$ (nm)	$\varepsilon_{326\ nm}$ ($cm^{-1}\ M^{-1}$)	$\lambda_{em,max}$ (nm)	ϕ (%)	τ (ms)
DATTA-Eu^{3+}	292, 326	2.03×10^4	612	0.25	1.08
BPTTA-Eu^{3+}	293,326	2.05×10^4	612	11.8	1.30

*All data were obtained in 0.05 M borate buffer of pH 7.4.

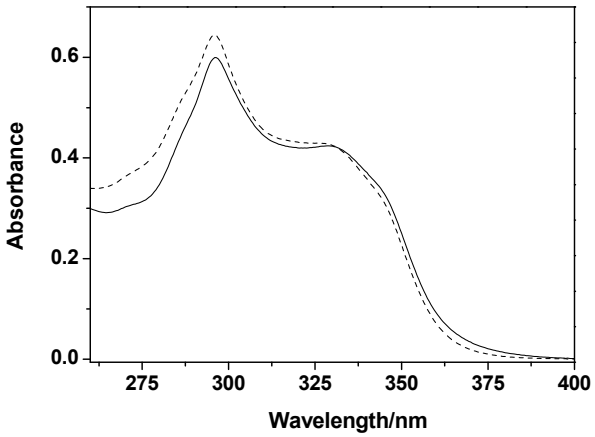


Fig. S1. Absorption spectra of DATTA-Eu^{3+} (20 μM, solid line) and BPTTA-Eu^{3+} (20 μM, dash line) in 0.05 M borate buffer of pH 7.4.

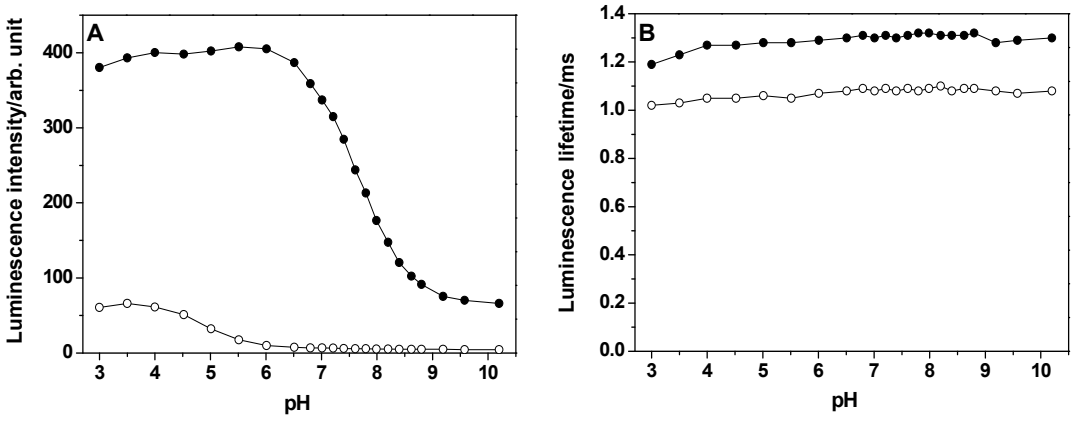


Fig. S2. Effects of pH on the luminescence intensities (A) and lifetimes (B) of DATTA-Eu^{3+} (1.0 μM, ○) and BPTTA-Eu^{3+} (1.0 μM, ●) in 0.05 M borate buffers with different pHs.

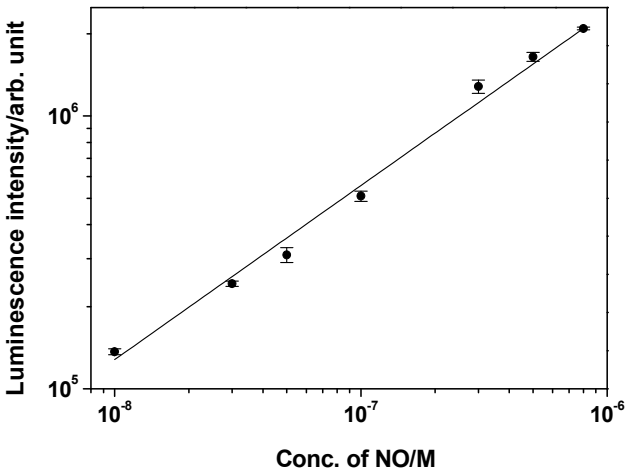


Fig. S3. Calibration curve for the time-gated luminescence detection of NO using DATTA-Eu^{3+} as a probe.

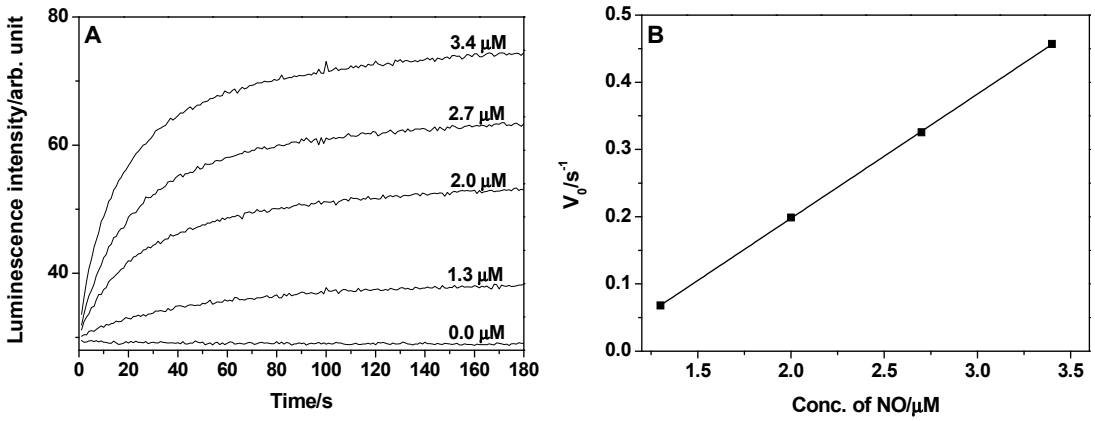


Fig. S4. (A) Reaction kinetic curves of DATTA-Eu^{3+} (2.0 μM) with different concentrations of NO. (B) The plot of initial rates (V_0) of the reaction of DATTA-Eu^{3+} with NO against the concentrations of NO.

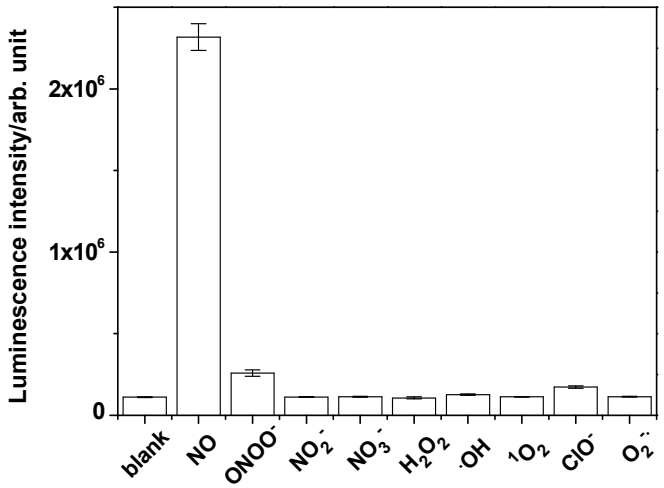
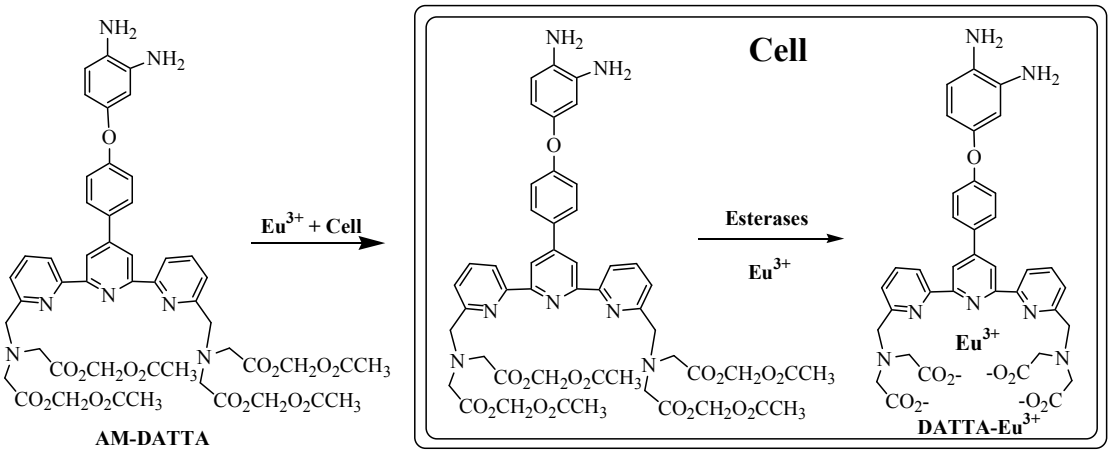


Fig. S5. Luminescence responses of DATTA-Eu^{3+} (0.1 μM) to different ROS and RNS (10 μM) in 0.05 M borate buffer of pH 7.4.



Scheme S2. Schematic diagram of the cell loading process of DATTA-Eu^{3+} chelate.

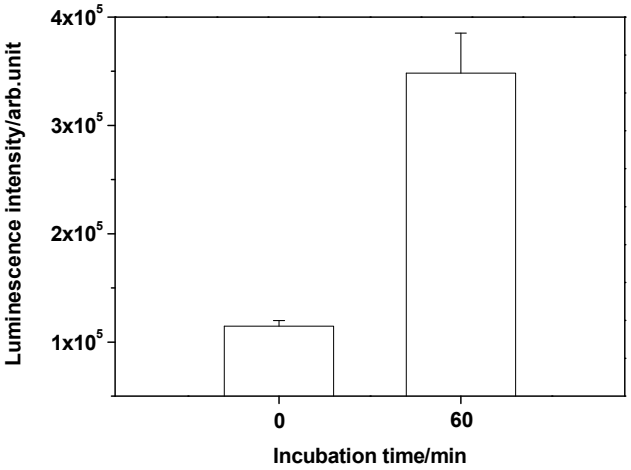


Fig. S6. Time-gated luminescence intensities of the solution used for incubating 50 mg of fresh *onion* inner-layer epidermal peels (2.0 mL of 0.05 M borate buffer of pH 7.4 containing 0.1 μM DATTA-Eu^{3+}) at different incubation times.

References

[S1] B. Song, G. L. Wang, M. Q. Tan, J. L. Yuan, *J. Am. Chem. Soc*. 2006, **128**, 13442-13450.

[S2] L. C. Green, D. A. Wagner, J. Glogowski, P. L. Skipper, J. S. Wishnok, S. R. Tannenbaum, *Anal. Biochem.* 1982, **126**, 131-138.

[S3] S. Miyamoto, G. R. Martinez, A. P. B. Martins, M. H. G. Medeiros, P. D. Mascio, *J. Am. Chem. Soc.* 2003, **125**, 4510-4517.

[S4] B. Lei, N. Adachi, T. Arai, *Brain Res. Protoc.* 1998, **3**, 33-36.

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

[S6] A. M. Held, D. J. Halko, J. K. Hurst, *J. Am. Chem. Soc.* 1978, **100**, 5732-5740.

[S7] K. M. Robinson, J. S. Beckman, in *Methods in Enzymology, Vol. 396* (Eds.: P. Lester, C. Enrique), Academic Press, **2005**, p. 207.

[S8] K. S. Gould, O. Lamotte, A. Klinguer, A. Pugin, D. Wendehenne, *Plant Cell Environ.* 2003, **26**, 1851-1862.

[S9] M. Moller, H. Botti, C. Batthyany, H. Rubbo, R. Radi, A. Denicola *J. Biol. Chem.* 2005, **280**, 8850-8854.