Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information for:

A Reversible Near-Infrared Fluorescence Probe for Reactive Oxygen Species Based on Te-rhodamine

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Methods

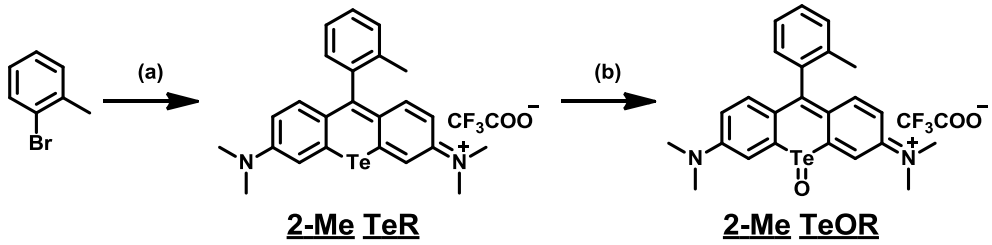
General Methods. General chemicals were of the best grade available, supplied by Tokyo Chemical Industries, Wako Pure Chemical, Aldrich Chemical Co., Alfa Aesar, Dojindo, GE Healthcare and Invitrogen, and were used without further purification. All solvents were used after appropriate distillation or purification. NMR spectra were recorded on a JEOL JNM-LA300 instrument at 300 MHz for ^{1}H NMR and at 75 MHz for ^{13}C NMR. Mass spectra (MS) were measured with a JEOL JMS-T100LC AccuToF using ESI. δ values are given in ppm relative to tetramethylsilane. HPLC analysis was performed on an Inertsil ODS-3 (4.6 × 250 mm) column (GL Sciences Inc.) using an HPLC system composed of a pump (PU-980, JASCO) and a detector (MD-2015 or FP-2025, JASCO). Preparative HPLC was performed on an Inertsil ODS-3 (10.0 × 250 mm) column (GL Sciences Inc.) using an HPLC system composed of a pump (PU-2080, JASCO) and a detector (MD-2015 or FP-2025, JASCO).

UV-Vis Absorption and Fluorescence Spectroscopy. UV-visible spectra were obtained on a Shimadzu UV-1650. Fluorescence spectroscopic studies were performed on a Hitachi F4500. The slit width was 2.5 nm for excitation and 5.0 nm for emission. The photomultiplier voltage was 700 V. Relative fluorescence quantum efficiency of Te-rhodamines was obtained by comparing the area under the emission spectrum of the test sample excited at 680 nm with that of a solution of Cy5.5 in PBS at pH 7.4, which has a quantum efficiency of 0.23.

Methods of experiments using living cells. HeLa cells were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% (v/v) fetal bovine serum, penicillin (100 units/mL) and streptomycin (100 μg/mL) in a humidified incubator containing 5% CO_2 gas. HL-60 cells were cultured in Roswell Park Memorial Institute (RPMI) medium supplemented with 10% (v/v) fetal bovine serum, penicillin (100 units/mL) and streptomycin (100 μg/mL) in a humidified incubator containing 5% CO_2 gas. HL-60 cells used in this experiment (Figure 4, Figure S11) were cultured in differentiation-inducing medium, RPMI medium containing 1.25% DMSO, for 5 days.[SR1] For spectrometry, HeLa cells and induced HL-60 cells were diluted to 2.5×10^5 cells/mL.

Synthesis and characterization

Scheme S1. Synthesis of 2-Me TeR and 2-Me TeOR.*



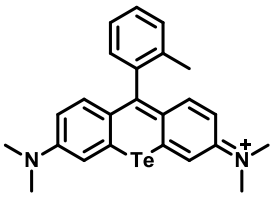
Reagents: (a) (i) *s*-BuLi/THF, – 78 °C (ii) Te-xanthone[SR2]/THF, – 78 °C to r.t. (iii) 2 N HCl, r.t., y. 68 %; (b) H_2O_2/MeOH, r.t., y. 20 %.

***2-Me TeR** and **2-Me TeOR** were each purified by reverse-phase HPLC as described in the following sections. The fractions containing the product were used to measure photophysical properties, except the molar extinction coefficient (Figure 1), to evaluate fluorescence response of **2-Me TeR** in the reaction with various reactive oxygen species (ROS) (Figure 2) and to test the reversibility of the reduction-oxidation reaction cycle (Figure 3). Powdered product obtained by freeze-drying was used to determine the extinction coefficient (Figure 1c) and for cell-based assay (Figure 4). See procedures/notes described below.

Synthetic procedures and characterizations

Elution in all HPLC analyses was done with a linear gradient (eluent, 0 min, 20 % CH_3CN/0.1 % TFA aq. ~ 20 min, 80 % CH_3CN/0.1 % TFA aq.; flow rate = 1.0 mL/min).

2-Me TeR



To a flame-dried flask flushed with argon, 2-bromotoluene (85.5 mg, 0.500 mmol) and anhydrous THF (5

mL) were added. The solution was cooled to – 78 °C, 1 M *s*-BuLi (0.500 mL, 0.500 mmol) was added, and the mixture was stirred for 30 min. At the same temperature, Te-xanthone[SR2] (10.0 mg, 0.0254 mmol) dissolved in anhydrous THF (5 mL) was slowly added, and the mixture was warmed to r.t. then stirred for 2 h. The reaction was quenched by addition of 2 N HCl and the mixture was stirred at r.t. for 10 min. Saturated $NaHCO_3$ was added, and the whole was extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4, and the solvent was evaporated. The residue was purified by preparative reverse-phase HPLC with a linear gradient [ODS: starting eluent 16% CH_3CN/0.1% TFA aq; final eluent 64% CH_3CN/0.1% TFA aq.; gradient duration 20 min; flow rate = 5 mL/min]. Fractions containing the product were combined and freeze-dried to afford **2-Me TeR** (as TFA salt, 10.1 mg, 0.0174 mmol, 68 % yield).

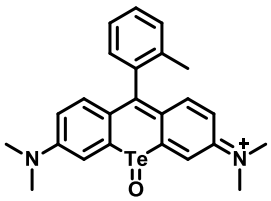
^{1}H-NMR (300 MHz, CD_3OD) δ: 1.94 (s, 3H), 3.23 (s, 12H), 6.91 (dd, 2H, *J* = 10.3, 2.9 Hz), 7.14 (d, 1H, *J* = 7.3 Hz), 7.38-7.55 (m, 5H), 7.90 (d, 2H, *J* = 2.9 Hz)

^{13}C-NMR (75 MHz, CD_3OD) δ: 19.4, 40.6, 116.2, 117.1, 122.6, 127.2, 130.1, 130.3, 131.6, 137.0, 140.1, 140.5, 141.0, 153.7, 165.7

HRMS (ESI-Tof) m/z Found 471.1076 M^+, calculated 471.1080 for $C_{24}H_{25}N_2Te$ (– 0.38 mmu)

t_R = 22.6 min.

2-Me TeOR



2-Me TeR (15.0 mg, 0.0258 mmol) was dissolved in MeOH (2 mL). Hydrogen peroxide (30-35%, Wako Pure Chemical Industries) was added dropwise (the volume of each drop was ~5 μL) with vigorous stirring at room temperature. When the color of the solution, initially deep blue, began to change to light green, the reaction mixture was immediately diluted with 3 mL of 0.1% TFA aqueous solution. The whole (5 mL) was purified by preparative reverse-phase HPLC with a linear gradient [ODS: starting eluent 16%

CH_3CN/0.1% TFA aq; final eluent 80% CH_3CN/0.1% TFA aq; gradient duration 20 min; flow rate = 5.0 mL/min]. Fractions containing the product were combined and freeze-dried to afford **2-Me TeOR** (as TFA salt, 3.1 mg, 0.00518 mmol, 20% yield).

^{1}H-NMR (300 MHz, CD_3OD) δ: 2.03 (s, 3H), 3.44 (s, 12H), 6.92 (dd, 2H, *J* = 9.5, 2.9 Hz), 7.14 (d, 1H, *J* = 8.0 Hz), 7.26 (d, 2H, *J* = 9.5 Hz), 7.37-7.55 (m, 3H), 8.14 (d, 2H, *J* = 2.9 Hz)

HRMS (ESI-Tof) m/z Found 487.1038 M^+, calculated 487.1029 for $C_{24}H_{25}N_2OTe$ (+ 0.88 mmu)

t_R = 13.4 min.

Notes

2-Me TeR or **2-Me TeOR** powder obtained by means of freeze-drying was used for NMR and MS analysis, and then to prepare 5 mM DMF stock solution for determining the extinction coefficient (Figure 1c), as well as for cell-based assay (Figure 4). On the other hand, combined reverse-phase HPLC fractions containing purified **2-Me TeR** or **2-Me TeOR** were used immediately, without further purification or concentration, as a stock solution for the other tests. The concentration of stock solutions was established by calculation from the molar excitation coefficient (1.0×10^5 M^{-1} cm^{-1} at 600 nm for **2-Me TeR**; 1.2×10^5 M^{-1} cm^{-1} at 669 nm for **2-Me TeOR**). Solutions appropriately diluted with buffer (usually containing less than 0.2% HPLC eluent) were used for Abs/Fl spectroscopic and photophysical studies (Figure 1, except determination of molar extinction coefficient), for tests of reactivity with various ROS (Figure 2) and for the reduction-oxidation cycle reversibility test (Figure 3). Reaction conditions with various ROS are described in the following section (Figure S3-S8) as well as in our previous reports. It is important to note that we utilized freshly prepared (within 3 hours) stock solutions for these studies, and the stock solutions were kept in the dark on ice to avoid artifacts arising from the formation of decomposition compounds.

Supporting Figures

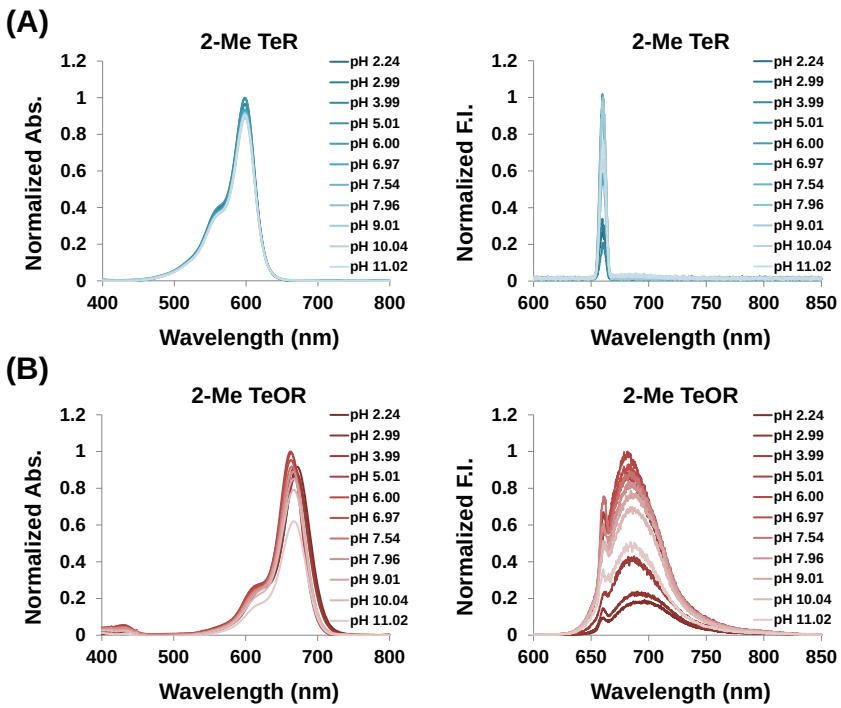


Figure S1. Normalized absorption and emission spectra of (A) 2-Me TeR and (B) 2-Me TeOR. Measurements were performed in sodium phosphate buffer at the indicated pH (pH 2.24 to pH 11.02). Excitation wavelength for fluorescence spectra was 660 nm.

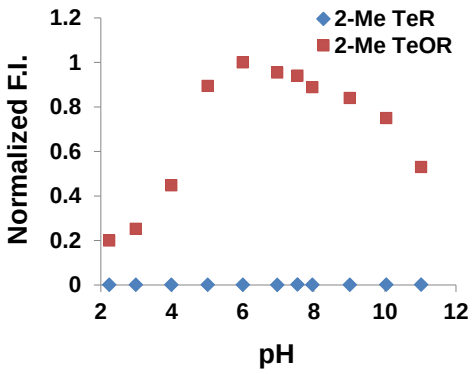


Figure S2. Normalized fluorescence intensity in sodium phosphate buffer at the indicated pH was plotted. 2-Me TeR was non-fluorescent in aqueous buffer at various pH values (pH 2.24 to pH 11.02). 2-Me TeOR was strongly fluorescent under physiological conditions (pH ~7.4).

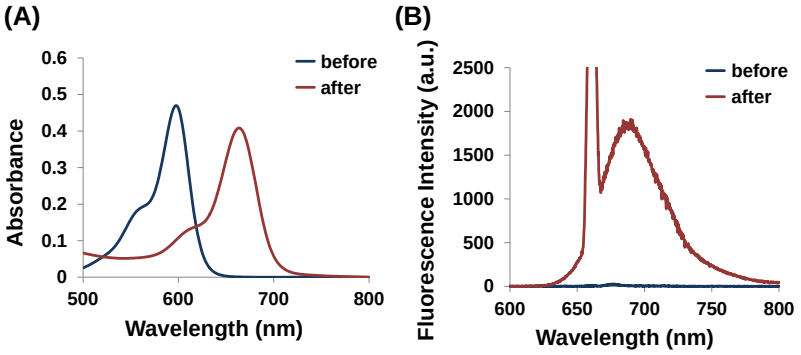


Figure S3. Reactivity of 2-Me TeR with $^{\bullet}OH$. Absorption (A) and fluorescence (B) spectra of 5 μM 2-Me TeR in PBS (pH 7.4) before and after addition of 500 μM ferrous perchlorate and 500 μM H_2O_2 are shown. Excitation wavelength for fluorescence spectra was 660 nm.

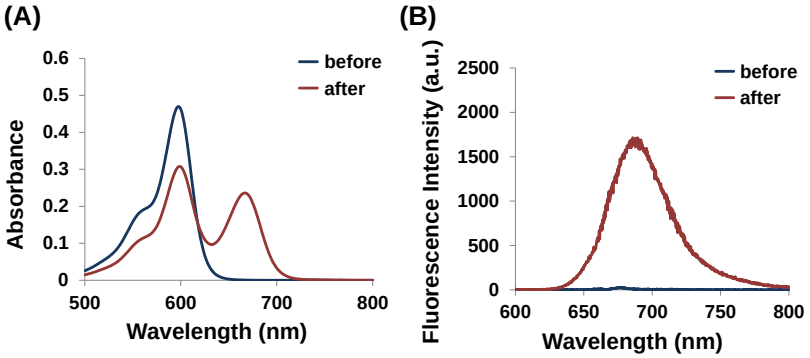


Figure S4. Reactivity of 2-Me TeR with $ONOO^-$. Absorption (A) and fluorescence (B) spectra of 5 μM 2-Me TeR in PBS (pH 7.4) before and after addition of 5 μM $ONOO^-$ are shown. Excitation wavelength for fluorescence spectra was 660 nm.

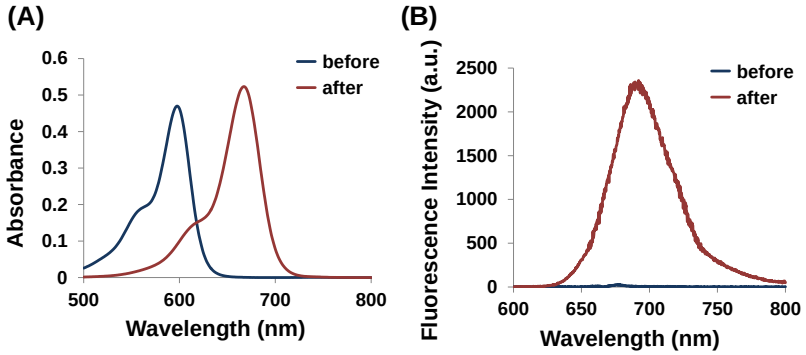


Figure S5. Reactivity of 2-Me TeR with $^{-}$OCl. Absorption (A) and fluorescence (B) spectra of 5 μM 2-Me TeR in PBS (pH 7.4) before and after addition of 5 μM NaOCl are shown. Excitation wavelength for fluorescence spectra was 660 nm.

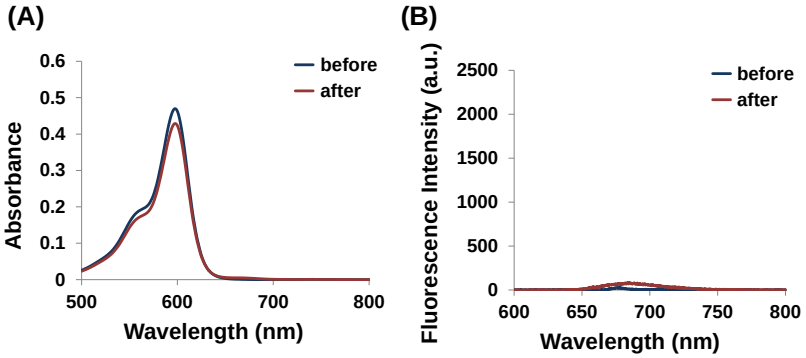


Figure S6. Reactivity of 2-Me TeR with $O_2^{-\bullet}$. Absorption (A) and fluorescence (B) spectra of 5 μM 2-Me TeR in PBS (pH 7.4) before and after addition of 100 μM KO_2 are shown. Excitation wavelength for fluorescence spectra was 660 nm.

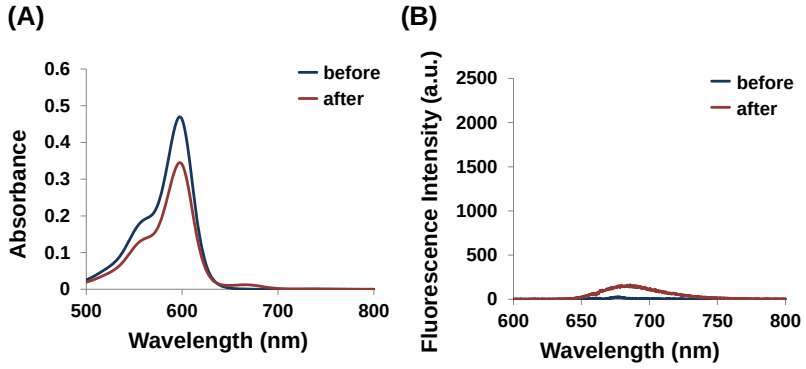


Figure S7. Reactivity of 2-Me TeR with H_2O_2. Absorption (A) and fluorescence (B) spectra of 5 μM 2-Me TeR in PBS (pH 7.4) before and after addition of 100 μM H_2O_2 are shown. Excitation wavelength for fluorescence spectra was 660 nm.

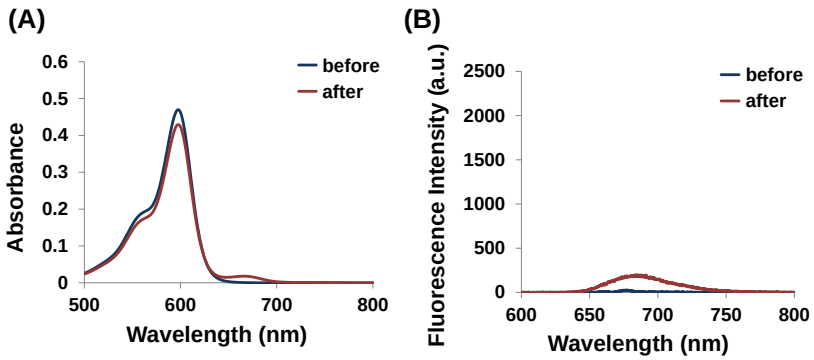


Figure S8. Reactivity of 2-Me TeR with $^{\bullet}NO$. Absorption (A) and fluorescence (B) spectra of 5 μM 2-Me TeR in PBS (pH 7.4) before and after addition of 100 μM NOC7 are shown. Excitation wavelength for fluorescence spectra was 660 nm.

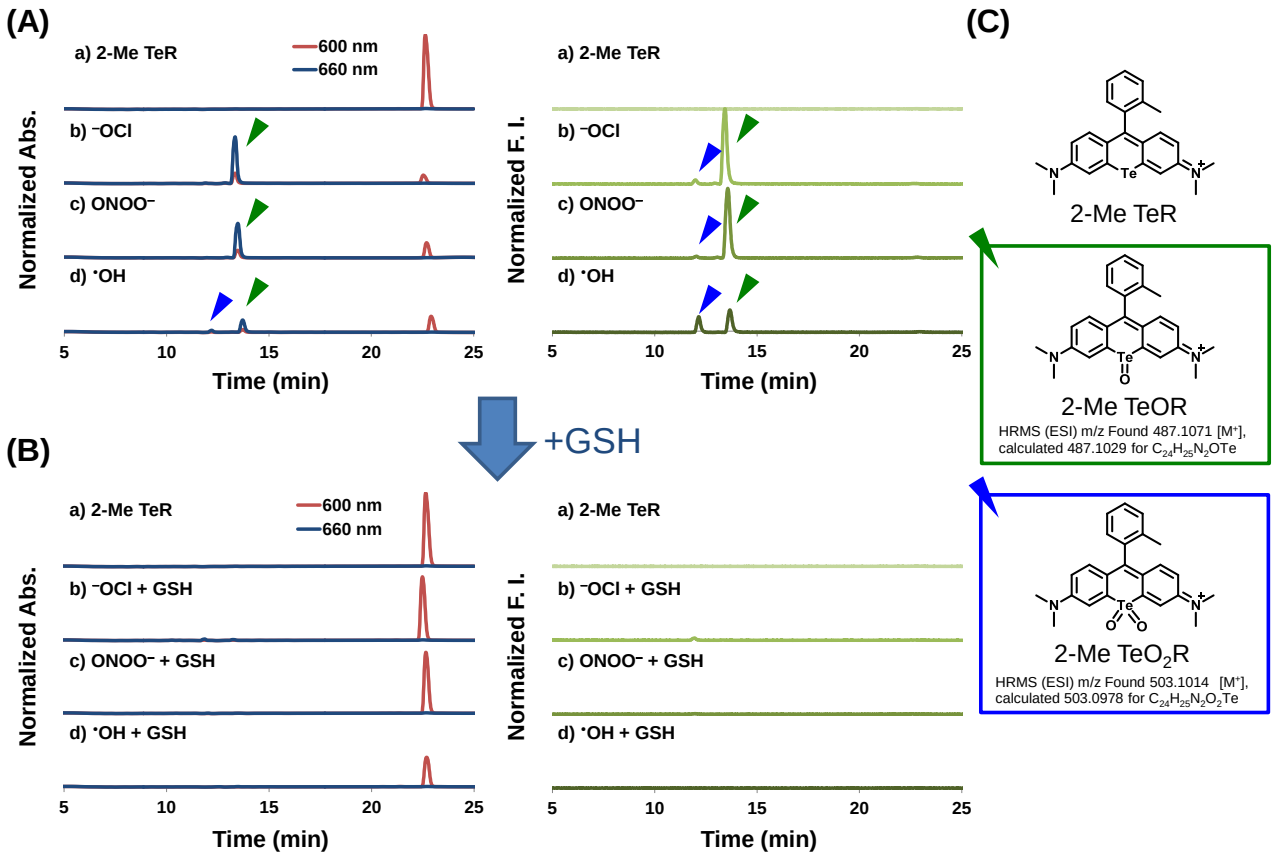


Figure S9. HPLC analysis of reactions of 10 μM 2-Me TeR with b) 10 μM $^{-}$OCl, c) 10 μM $ONOO^{-}$ and d) ·OH before (A) and after (B) the addition of 1 mM GSH. The ·OH was generated by Fenton reaction ($Fe(ClO_4)_2$: 20 μM, H_2O_2: 20 μM (final)). The reactions were performed in PBS (pH 7.4) at room temperature for 5 min. The generated products were identified by mass spectrometry and the identified structures are shown in (C). Under these conditions, 2-Me TeR was converted into 2-Me TeOR and also partly 2-Me TeO_2R. This result indicates that 2-Me TeO_2R was also reduced to 2-Me TeR by GSH. For fluorescence chromatograms, excitation and emission wavelengths were 660 and 680 nm, respectively.

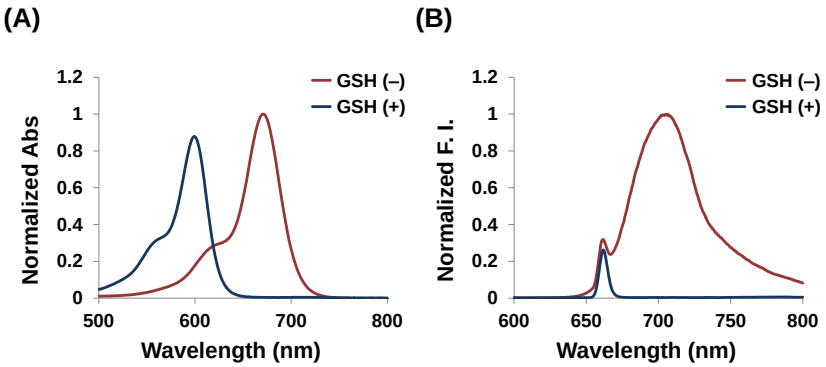


Figure S10. Reactivity of 2-Me TeOR with GSH. Normalized absorption (A) and fluorescence (B) spectra of 5 μM 2-Me TeOR in PBS (pH 7.4) in the absence and presence of 100 μM GSH (reduced form) are shown. Excitation wavelength for fluorescence spectra was 660 nm.

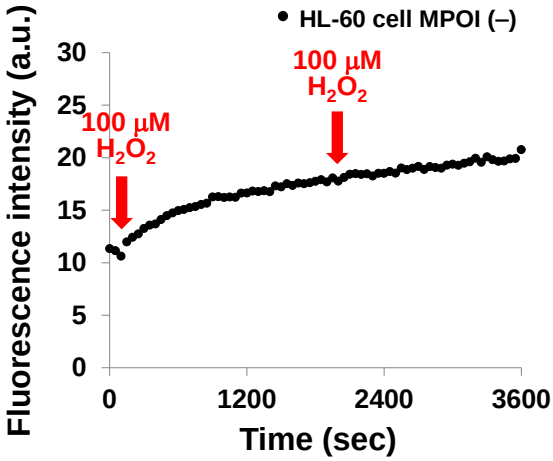


Figure S11. Time courses of the change in fluorescence intensity observed with 5 μM APF^{SR3} for H_2O_2-stimulated HL-60 cells (2.5×10^5 cells/mL) in HBSS at 37 °C. 100 μM H_2O_2 (final concentration) was added at 100 sec and 2000 sec. The fluorescence intensity was measured at 520 nm with excitation at 490 nm.

Supporting references

SR1 S. J. Collins, F. W. Ruscetti, R. E. Gallagher, and R. C. Gallo, *Proc. Natl. Acad. Sci. U.S.A.* 1978, **75,** 2458.

SR2 B. Calitree, D. J. Donnelly, J. J. Holt, M. K. Gannon, C. L. Nygren, D. K. Sukumaran, J. Autschbach, and M. R. Detty, *Organometallics* 2007, **26**, 6248.

SR3 K. Setsukinai, Y. Urano, K. Kakinuma, H. J. Majima, and T. Nagano, *J. Biol. Chem.* 2003, **278**, 3170.