Supporting Information

For

Reaction-based Turn-on Fluorescent Probes with Magnetic Responses for Fe^{2+} Detection in Live Cells

Siddhartha Maiti,[a,b,c] Ziya Aydin,[c,d] Yi Zhang,[a,c] and Maolin Guo,[a,b,c,d*]

[a] Department of Chemistry and Biochemistry; University of Massachusetts Dartmouth, 285 Old Westport Road, Dartmouth, MA02747 (USA)
[b] Biomedical Engineering & Biotechnology PhD Program, University of Massachusetts Dartmouth, 285 Old Westport Road, Dartmouth, MA02747(USA)
[c] UMass Cranberry Health Research Center, University of Massachusetts Dartmouth, 285 Old Westport Road, Dartmouth, MA02747(USA)
[d] Department of Chemistry, University of Massachusetts Amherst, 710 North Pleasant Street, Amherst, MA 01003 (USA)

*mguo@umassd.edu

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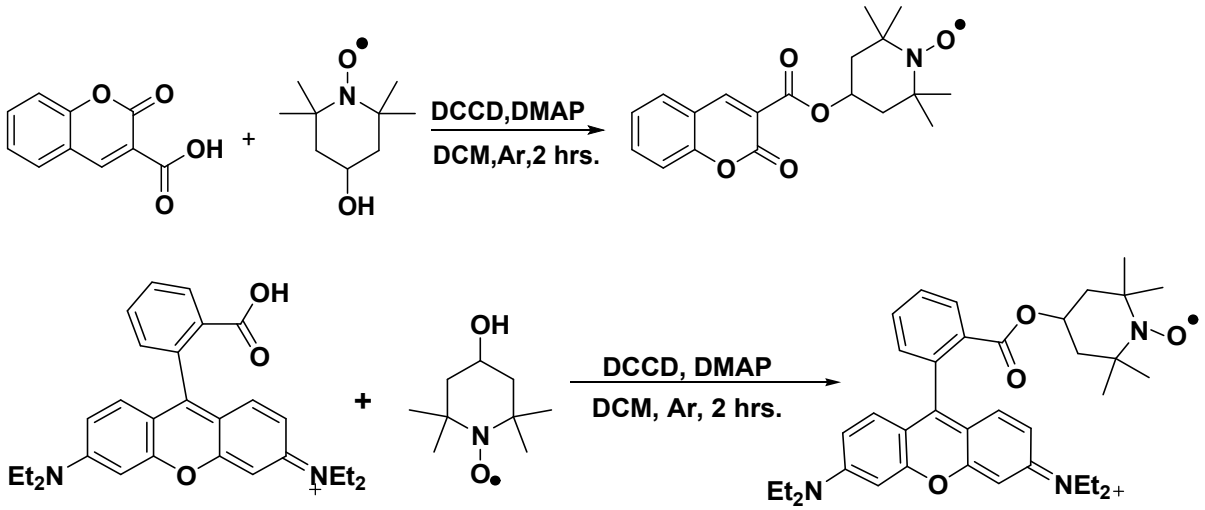
1. Materials and instruments

Rhodamine B, 4-hydroxy TEMPO, N,N'-Dicyclohexylcarbodiimide (DCC), and 4-Dimethylaminopyridine(DMAP) were purchased from Sigma-Aldrich. The other chemicals and the solvents used in the experiments were purchased commercially.

A stock solution of **Rh-T**(1 mM) was prepared in DMF. The solution of **Rh-T** was diluted to 6 μM with MOPS buffer (10 mM, pH 7.3). In selectivity experiments, the test samples were prepared by appropriate amount of metal ion stock into 1 ml solution of **Rh-T (**6 μM**).** ESI-MS analyses were performed using a Perkin-Elmer API 150EX mass spectrometer. UV/Vis spectra were recorded on a Perkin-Elmer Lambda 25 spectrometer at 293 K. Fluorescence spectra was recorded on a Perkin-Elmer LS55 luminescence spectrometer at 293 K. The pH measurements were carried out on a Corning pH meter equipped with a Sigma-Adrich micro combination electrode calibrated with standard buffer solutions. EPR spectra were recorded on a Bruker ECS106 spectrometer at room temperature.

2. Synthesis of Cou-T and Rh-T



Scheme S1. Synthesis of Cou-T (top) and Rh-T(bottom)

Rhodamine B was esterified with 4-hydroxy-TEMPO in the presence of 1,3-dicyclohexanecarbodiimide and 4-(dimethylamino) pyridine in CH_2Cl_2 under argon atmosphere for 2 h.

More details; 4-Hydroxy TEMPO (0.2 g, 1.16 mmol), Rhodamine B (0.25 g, 0.53 mmol), and DMAP (1.5 mg) were stirred in dry CH_2Cl_2 (5 mL) under nitrogen atmosphere. In the separate flask, DCC was diluted with dry CH_2Cl_2 (5 mL) and pyridine (0.1 g). This solution was then added by a syringe to the reaction mixture and stirred for 2 hours at room temperature under nitrogen atmosphere. The solvent was removed under reduced pressure to give the crude product, which was purified by silica gel flash chromatography using Hexane to Hexane/EtOAC (0 to

1;1) as eluent to afford the compound **Rh-T** (0.22 g, yield 67%). ESI-MS: found: *m/z* = 597.4 $[M]^+$ (without Cl^-), calcd for $C_{37}H_{47}N_3O_4^{+\cdot}$ = 597.7.

3. Quantum Yield

Stock solutions of 40 μM Rh-T, 40 μM Rhodamine B (standard), and 40 μM Rh-T +Fe^{2+} were prepared in EtOH. Dilutions of Rh-T, Rhodamine B, Rh-T+Fe were prepared in EtOH at concentrations such that their absorbance at 510 nm equaled 0.1, 0.2, 0.3, 0.4, and 0.5 μM. Excitation was performed at 510 nm and collected emission was normalized to the EtOH blank and then integrated from 530 to 700 nm. A plot of the integrated fluorescence intensity vs. the absorbance at 510 nm for each concentration was prepared and the positive slope of the linear fit was calculated. The data were compared to the rhodamine standard using the following equation, where Φ_R is the quantum yield of the standard (0.97), Grad is the slope of the absorbance vs. emission line found for each compound, GradR is the slope found for the Rhodamine standard, n is the refractive index of the sample solutions (1.33) and n_R is the refractive index of the fluorescein solution (1.33):

$$\Phi = \Phi_R \, (\mathrm{Grad}/\mathrm{Grad}_R) \, (n^2/n_R^2)$$

$$\Phi_{Rh\text{-}T} = 0.134 \; \Phi_{Rh\text{-}T + Fe(II)} = 0.280$$

4.Cell culture experiments: Human fibroblast ws1 cells were grown at 37 ° C in a humid atmosphere of 5% CO_2 atmosphere in eagle's minimum essential medium (EMEM,ATCC) supplemented with 10% fetal bovine serum (FBS, ATCC). Cultures were divided into 1:2 every 48 h to an approximate cell density of 1.21 million cells/ml and used for experiments after 24 h.

5. Confocal fluorescence imaging experiments:

A Zeiss LSM 710 laser-scanning confocal microscope system was used for cell imaging experiments. 40x oil-immersion objective lens were used to perform all the experiment. For imaging with the Rh-T sensor, excitation wavelength of the laser was 543 nm and emission were integrated over the range 547-703. For images with Mito Tracker Green FM, Lyso Tracker Blue DND-22, excitation wavelengths were set following the protocol provided by the manufacturer. Emission were integrated at 492-548 (Mito Tracker), 409-484 nm (Lyso Tracker) respectively.

ws1 cells, at an approximately density of 1.2 million/ml in complete EMEM medium, were incubated with 100 μM ferrous ammonium sulfate (FAS, $Fe(NH_4)_2(SO_4)_2$ from 10 mM stock solution) for overnight at 37 ° C in a humid atmosphere of 5% CO_2 atmosphere and then the cells were washed with fresh EMEM medium to remove excess Fe^{2+}.Then cells were incubated with Rh-T(1 μM, from 500 μM stock solution in DMF) at 37 ° C for 30 min and then cells were washed with EMEM media and then imaged. In addition, some cells were treated firstly with 100 μM Fe(II) overnight and then the cells were washed with fresh EMEM medium and then incubated with 1 mM 2,2'-bipyridyl (Bpy) at 37 °C for 30 min for chelation experiment and then sensor was added followed by washing with the media and then imaging was done. Some cells were treated with 1 mM 2,2'-bipyridyl alone and subsequently the sensor was added and then imaged. Controls were imaged without Fe(II) and 2,2'-bipyridyl incubation.

6. EPR experiment:

For EPR experiment, we treated the cells with Fe(II)/Bpy/sensor exactly the same way as we did for confocal experiment. After the incubation, cells were harvested by trypsinization and then were centrifuged. Aliquots of the concentrated cell pellets were immediately transferred to bottom-sealed Pasteur pipettes and the EPR spectra were recorded on a Bruker escan spectrometer at room temperature. Instrument settings: microwave frequency, 9.75 GHz; microwave power, 12.17 mW; sweep width, 100 G; modulation amplitude, 3.06 G; time constant, 5.12 ms; conversion time, 5.12 ms; sweep time, 2.62 s.

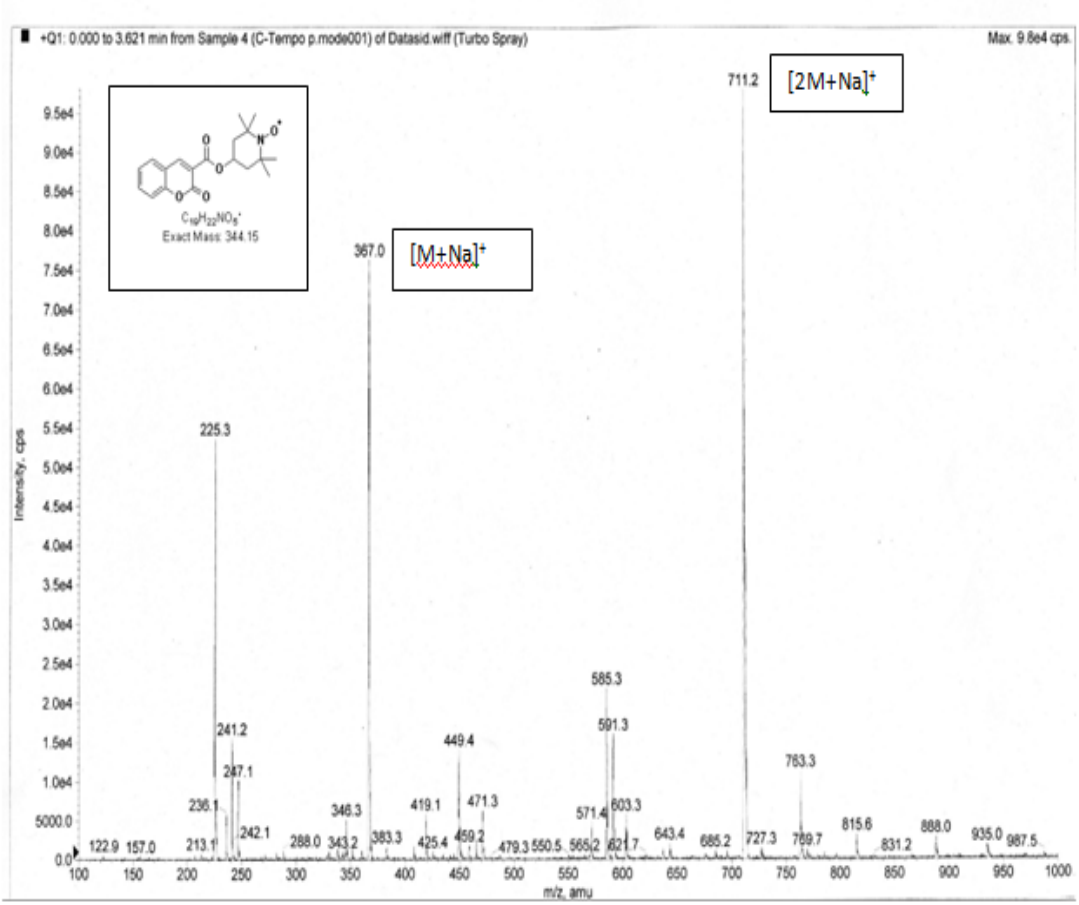


Figure S1. ESI-MS spectrum of Cou-T.

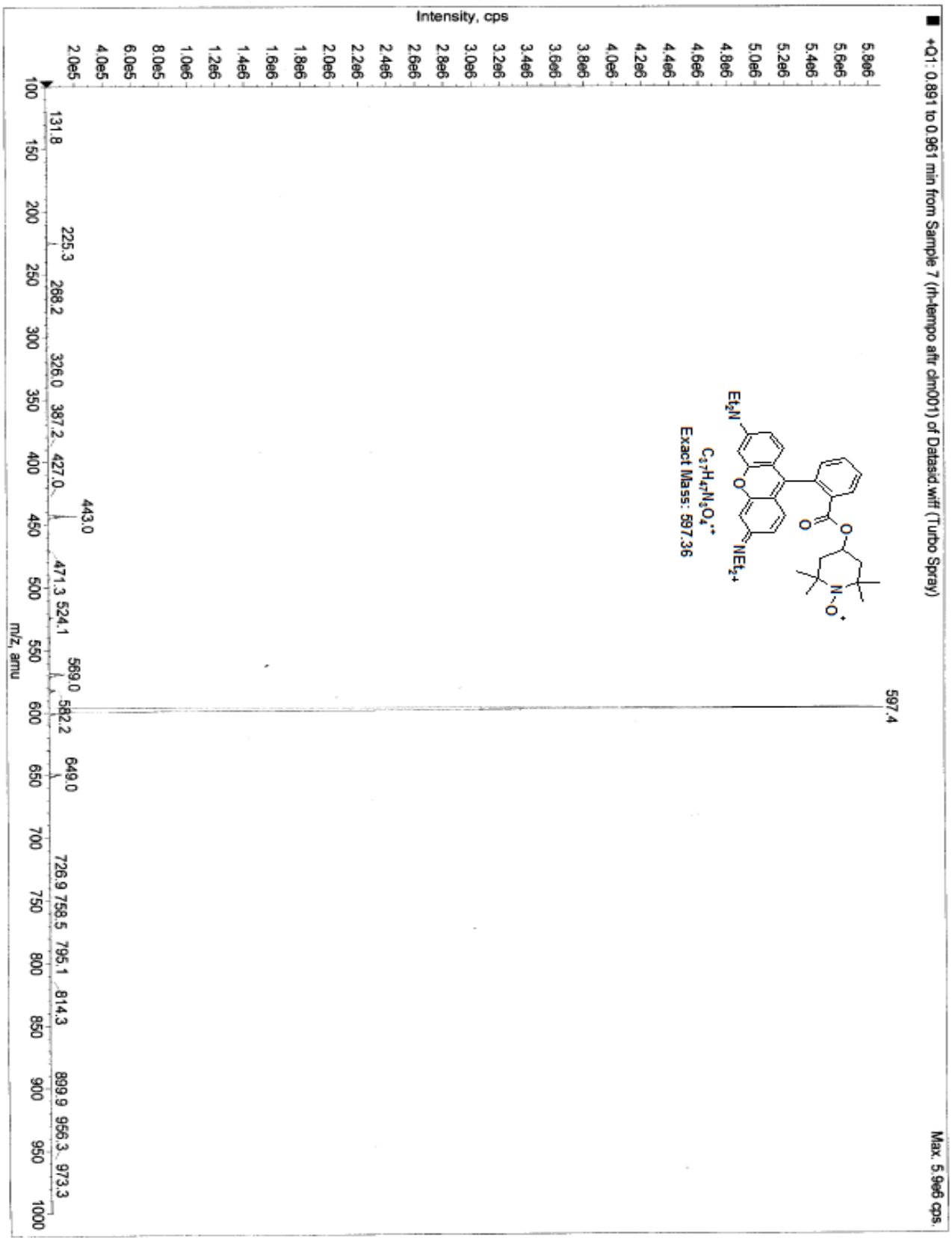


Figure S2. ESI-MS spectrum of Rh-T.

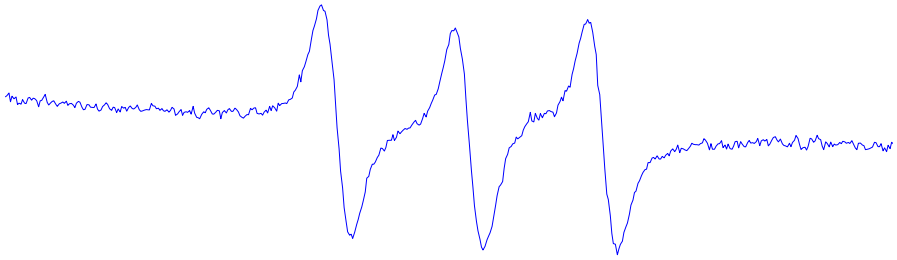
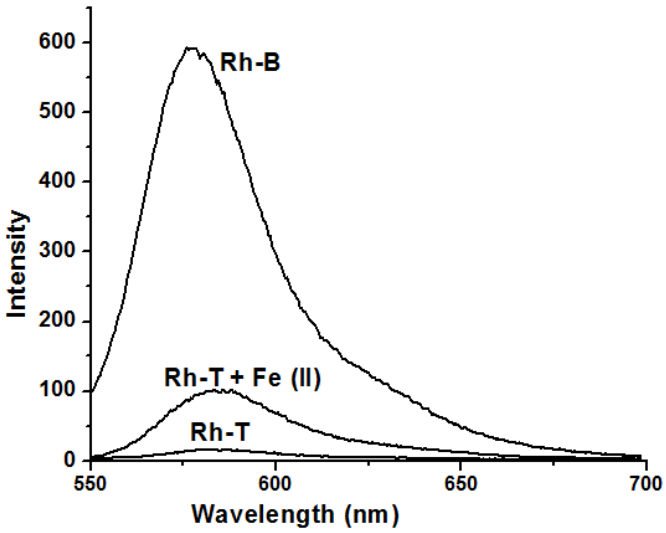


Figure S3. EPR spectrum of Rh-T (6 μM) displays the characteristic TEMPO radical pattern. The EPR spectrum of Cou-T displays the same pattern.



FigureS4. Fluorescence Intensity of Rh-B (rhodamine B) (1 μM), Rh-T+Fe^{2+} (1 μM), and Rh-T (1 μM**)**

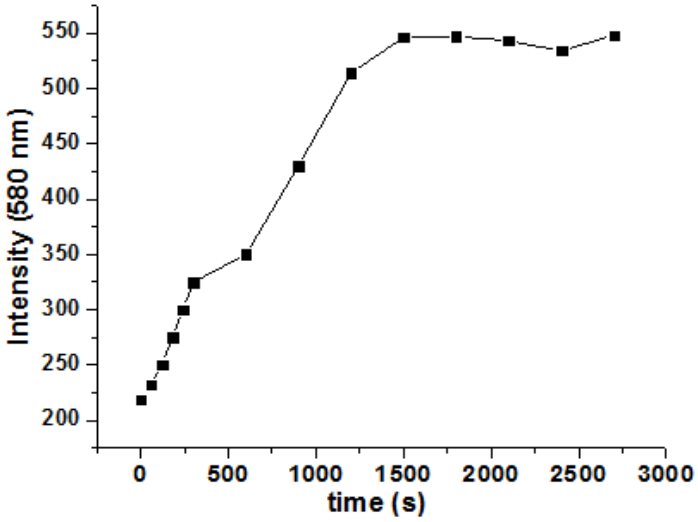


Figure S5. Time course plot of fluorescence intensity at 580 nm for the reaction of Rh-T and 20 μM Fe $(NH_4)_2(SO_4)_2$in MOPs buffer (10 mM, pH 7.3).

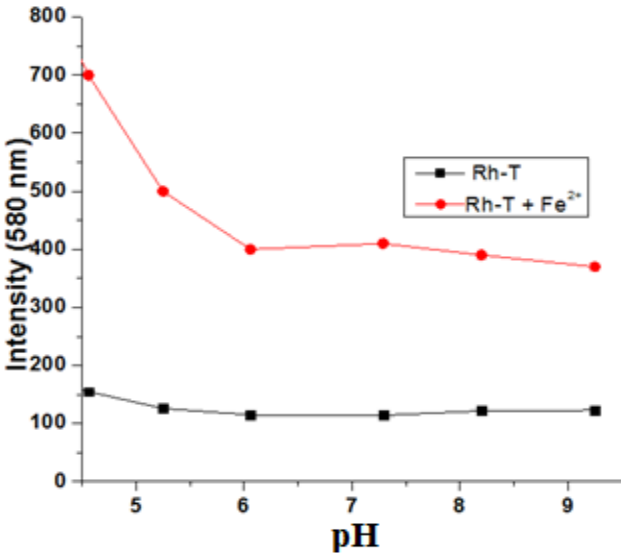


Figure S6. Variation of fluorescent intensity of Rh-T and Rh-T + Fe^{2+} (6 μM each) at various pH values in water. pH was adjusted with HCl and NaOH**.**

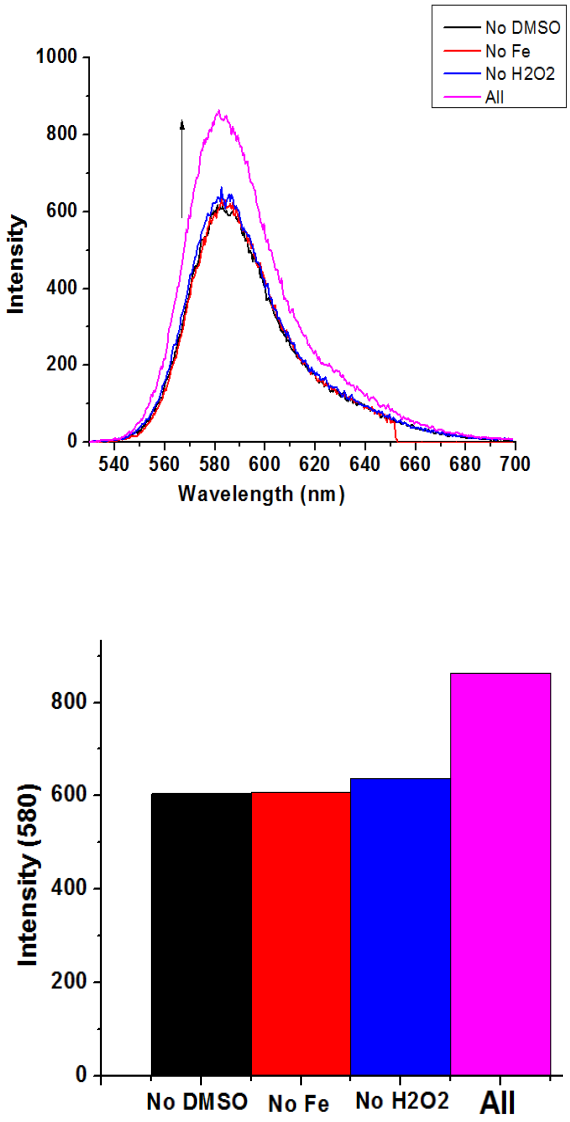


Figure S7. Fluorescence responses of Rh-T to H_2O_2, Fe (II)-EDTA and hydroxyl radicals (All, the Fenton system). Reaction mixture contained 10 µM H_2O_2, 70 µM Fe (II)-EDTA, 0.1 M DMSO and 3 µM Rh-T in 10 mM KPB buffer solution, pH 7.4..

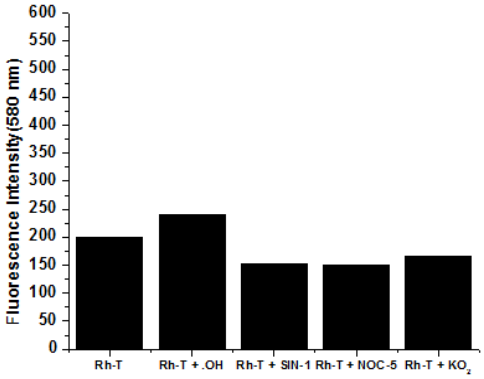


Figure S8. Fluorescence response of Rh-T (6 **μ**M) in reaction with 50 μM KO_2, 100 μM NOC-5, and 100 μM SIN-1. Experiment was carried out at 25° C in 10 mM KPB buffer (pH 7.3) with excitation at 510 nm.

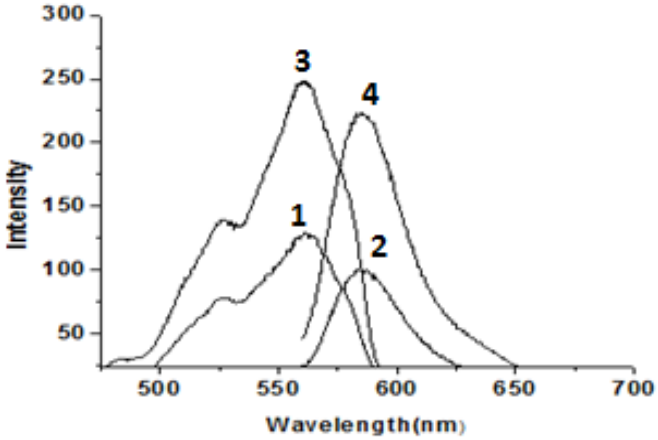


Figure S9. The excitation (lines 1 and 3) and emission spectra (lines 2 and 4) of the Rh-T (2.5 μM) reacting with or without ascorbic acid (25 μM) for 2 h in water (pH 5.3).

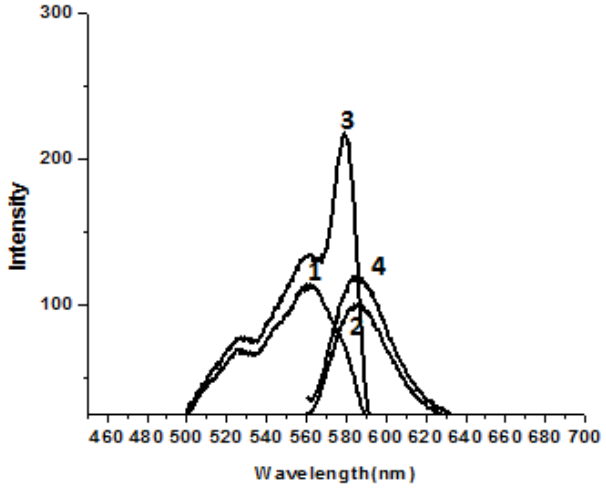


Figure S10. The excitation (lines 1 and 3) and emission spectra (lines 2 and 4) of the Rh-T (2.5 μM) reacting with or without ascorbic acid (25 μM) for 2 h in MOPS (pH 7.3**).**

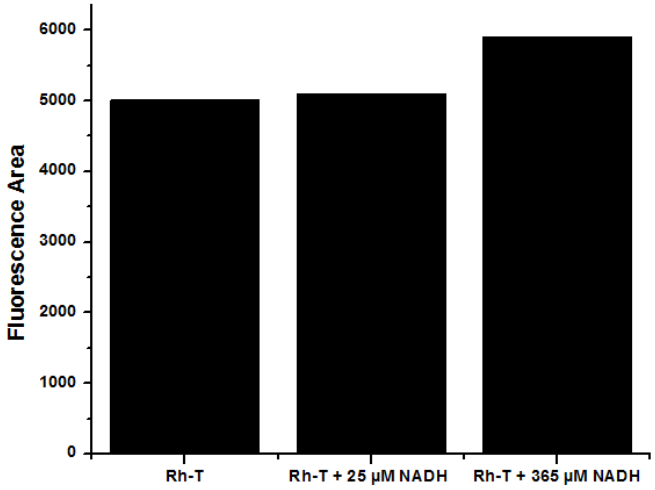


Figure S11. Fluorescence response of Rh-T(1 μM) in reaction with nicotinamide adenine dinucleotide (NADH). Experiments were carried out at 25° C in 10 mM MOPS buffer (pH 7.3) with excitation at 510 nm**.**

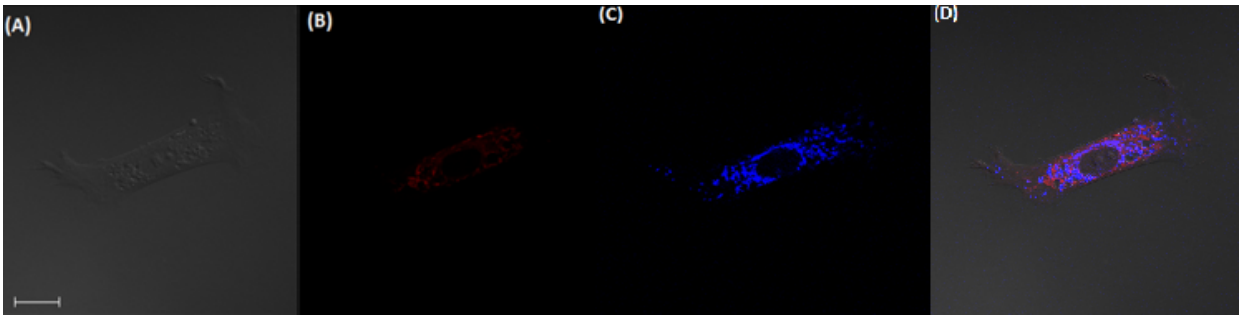


Figure S12. Representative confocal images of intracellular co-localization studies of 1 μM Rh-T incubated with live human ws1 fibroblast cells co-labeled with LysoTracker Blue DND-22 (50 nM). (A) DIC image of cells with 20 μm scale bar; (B) Rh-T fluorescence collected at 547-703 nm (red); (C)LysoTracker fluorescence collected at 409-484 nm (blue); (D) DIC image of (A) and fluorescence images of (B) and (c) were merged together.

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