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Single-layer MnO_2 nanosheets quenched fluorescence ruthenium complexes for sensitive detection of ferrous iron

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EXPERIMENTAL SECTION

Materials. Tris(2,2'-bipyridyl)dichlororuthenium(II) hexahydrate ($Ru(bipy)_3^{2+}$) and 3-[4,5-dimethylthialzol-2-yl]-2,5-diphenyltetrazolium bromid (MTT) were purchased from Sigma-Aldrich (USA), manganese chloride tetrahydrate ($MnCl_2•4H_2O$), tetramethylammonium hydroxide pentahydrate (TMA•OH) and hydrogen peroxide (H_2O_2, 30 wt%) were purchased from Alfa Aesar (China). Dimethyl sulfoxide (DMSO) was obtained from Xilong Reagent Company (Guangdong, China). Sodium chloride (NaCl), potassium chloride (KCl), Zinc chloride ($ZnCl_2$), ferric chloride ($FeCl_3$), chromium chloride hexahydrate ($CrCl_3•6H_2O$), cobalt sulfate ($CoSO_4•7H_2O$), sulfate ($CuSO_4•5H_2O$), magnesium sulphate ($MgSO_4•7H_2O$), cupric lead nitrate ($Pb(NO_3)_2$) and ferrous chloride ($FeCl_2$) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Ferrous sulfate tablet was from Huanghai Pharmaceutical Co., Ltd. (Shanghai, China). All chemicals were of analytical grade and used as received without further purification. All solutions were prepared using ultrapure water (18.2 MΩ·cm from Millpore purification system).

Apparatus and characterization. Transmission electron microscopy (TEM) measurements carried out on a F20 field-emission transmission electron microscope and an accelerating voltage of 200 KV. Atomic force microscopy (AFM) images were taken using a Multimode 8 (Bruker, USA). Zeta potential and DLS measurements were analyzed at 25°C on a Nano ZS90 laser particle analyzer (Malvern Instruments, UK), which equipped with a fixed scattering angle of 90° He-Ne laser (633 nm). UV-vis transmission spectra were collected using a UV-2600 UV-vis spectrometer (Shimadzu, Japan). Fluorescence measurements were performed on a Hitachi Model F-7000 Fluorometer (Hitachi Co., Ltd., Japan) with a 1 mm×10 mm quartz cuvette containing 100 μL of solution at room temperature. The samples were excited at 452 nm in steps of 1 nm. Ex slit and Em slit width were 10 nm (700 V PMT). The MTT results were obtained on a Benchmark Plus, Biorad Instruments Inc, Japan. The confocal laser scanning microscopy (CLSM) images were taken by a Fluoview FV500, Olympus.

Preparation of single-layer MnO_2 nanosheets. The single-layer manganese dioxide

nanosheets were prepared according to previous reports.[1] The bulk MnO_2 nanosheets were first synthesized by adding 0.3 M $MnCl_2$•$4H_2O$ aqueous solution (10 mL) into the mixture (20 mL) containing 0.6 M TMA•OH and 3.0 wt% H_2O_2 within 15 s and were continuously stirred vigorously overnight in the open air at room temperature. Upon mixed $MnCl_2$•$4H_2O$ with mixture of TMA•OH and H_2O_2, a dark brown suspension was formed, immediately, indicating that Mn^{2+} was oxidized to Mn^{4+}. After that, the as-prepared bulk manganese dioxide was collected by centrifugation at 10000 rpm for 10 minutes, washed with alcohol and water for several times and removed the residual solvent by cold drying the solid under high vacuum at -60 °C for 12. To prepare 1 mg mL^{-1} the single-layer MnO_2 nanosheets solution, 10 mg MnO_2 solid was dispersed in 10 mL ultrapure water and was degraded by ultrasonic cleaning machine and ultrasonic cell crasher.

Fluorescence quenching of the single-layer MnO_2 nanosheets toward $Ru(bipy)_3^{2+}$. The fluorescence suppression of $Ru(bipy)_3^{2+}$ by MnO_2 nanosheets was carried out by mixing various concentrations MnO_2 nanosheets (0-120 μg mL^{-1}) and $Ru(bipy)_3^{2+}$ (50 μM) reaching a total volume of 200 μL for each sample. Then, the fluorescence measurement was performed.

Analysis of Fe^{2+} in aqueous solutions. For fluorescent sensing detection of $FeCl_2$ solution, the fluorescence probe based on MnO_2-$Ru(bipy)_3^{2+}$ nanocomplex was first prepared by hybridized MnO_2 nanosheets (60 μg mL^{-1}) with $Ru(bipy)_3^{2+}$ (5 μM), and then various concentrations of $FeCl_2$ (0 to 2000 μM) were added into MnO_2-$Ru(bipy)_3^{2+}$ nanocomplex, respectively, reaching a total volume of 200 μL for each sample. The mixtures were incubated at room temperature for 5 min and then the fluorescence detections were performed.

Selectivity of the fluorescence probe. The selectivity of the fluorescence probe toward Fe^{2+} was inspected by using other common metal salt solutions as controls, including Na^+, K^+, Zn^{2+}, Fe^{3+}, Cr^{3+}, Co^{2+}, Cu^{2+}, Mg^{2+}, Pb^{2+}. Different common metal ion and Fe^{2+} were added into fluorescence probe solutions, respectively, reaching a total volume of 200 μL for each sample which including BSA (100 μg mL^{-1}). After being incubation with Fe^{2+} (100 and 500 μM), and other common metal salt solutions

(1 mM for each) for 5 min at room temperature, fluorescence intensities were detected.

Analysis of Fe^{2+} in the ferrous sulphate tablets. For fluorescent sensing of Fe^{2+} in anti-anemic pharmaceutical ferrous sulphate tablets, the coating tablet of ferrous sulphate tablets was first wiped off. Then, the solution of Fe^{2+} was prepared by dissolving the ferrous sulphate tablets into appropriate amount water. The detection way was same as the analysis of Fe^{2+} in aqueous solutions. The fluorescence intensity was detected and the concentration of Fe^{2+} was calculated according to the calibration curve.

Cell culture and cytotoxicity. HeLa cells were cultured in RPMI media with 10% fetal bovine serum, penicillin (100 U mL^{-1}), and streptomycin (100 mg mL^{-1}) at 37 °C in a 5% CO_2 incubator. Cell viability was measured by MTT assay. For the cytotoxicity assay of MnO_2-$Ru(bipy)_3^{2+}$ nanocomplex, HeLa cells were cultured in 96-well plates (200 μL, 7 × 10^3 cells per well). After grown for 24 h, the cells were treated with 200 μL of cell medium containing various concentrations of MnO_2-$Ru(bipy)_3^{2+}$ nanocomplex (0-120 μg mL^{-1}) and incubated for 24 h. At the preassigned time, the cell medium was then removed, and the cells were washed for with cell culture media for one time. Then, 180 μL fresh cell medium and 20 μL MTT solution (0.5 mg mL^{-1}) were added for another 4 h. After that, the medium was removed away and 150 μL of DMSO was added into each well to dissolve the precipitated formazan violet crystals at 37 °C for 10 min. The absorbance was measured at 490 nm by a multidetection microplate reader. Experiments were repeated in triplicate, and the error bars represent the standard derivations

Analysis of Fe^{2+} in living cells. For the confocal imaging analysis, HeLa cells were plated at around 60-70% confluency for 24 h before imaging experiments in 35-mm culture dishes. Prior to imaging experiments, the HeLa cells were treated with NEM (500 μM) for 30 min to decrease GSH concentration in living cells, and for Fe^{2+} analysis, Fe^{2+} (500 μM) was incubated with the HeLa cells treated with NEM for 2 h in RPMI media without fetal bovine serum. The cells were washed for three times with cell culture media and incubated with MnO_2-$Ru(bipy)_3^{2+}$ nanocomplex (60 μg mL^{-1}) for 3 h. The cell lines were further washed with cell culture media and

subsequently imaged at ambient temperature.

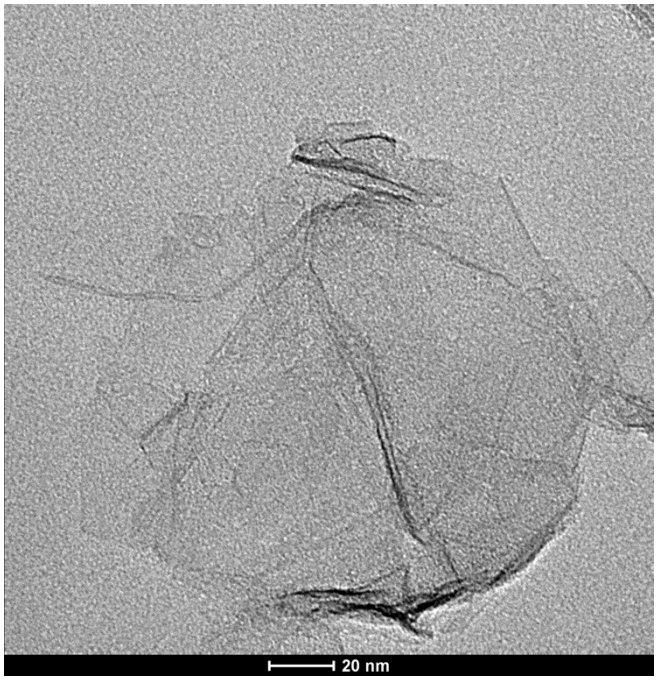

Fig. S1 TEM image of MnO_2 nanosheet.

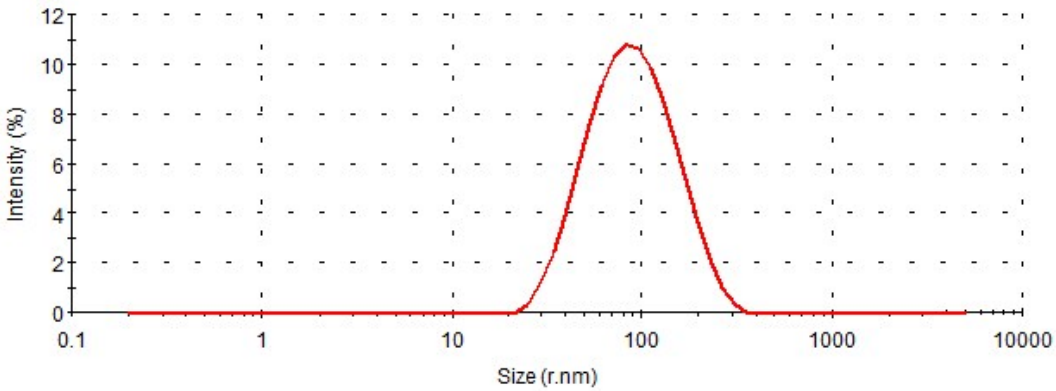

Fig. S2 DLS result of MnO_2 nanosheets.

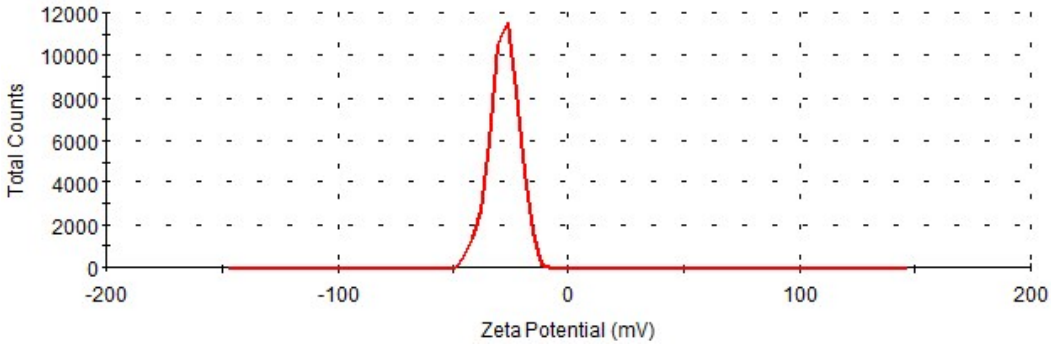

Fig. S3 Zeta potential of MnO_2 nanosheets (-27.8 mV).

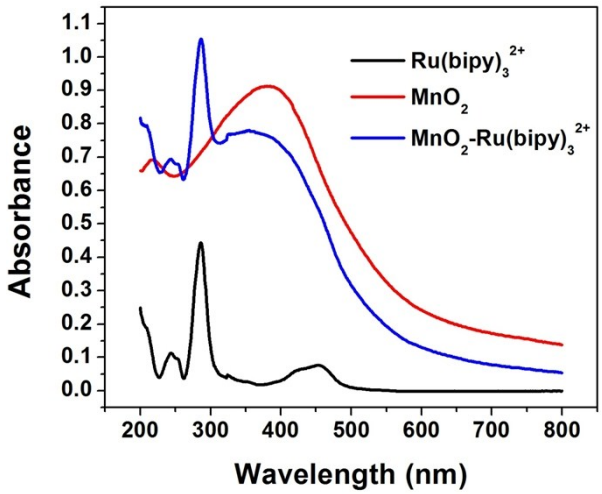

Fig. S4 UV-vis spectra of $Ru(bipy)_3^{2+}$, MnO_2 nanosheets, and MnO_2-$Ru(bipy)_3^{2+}$.

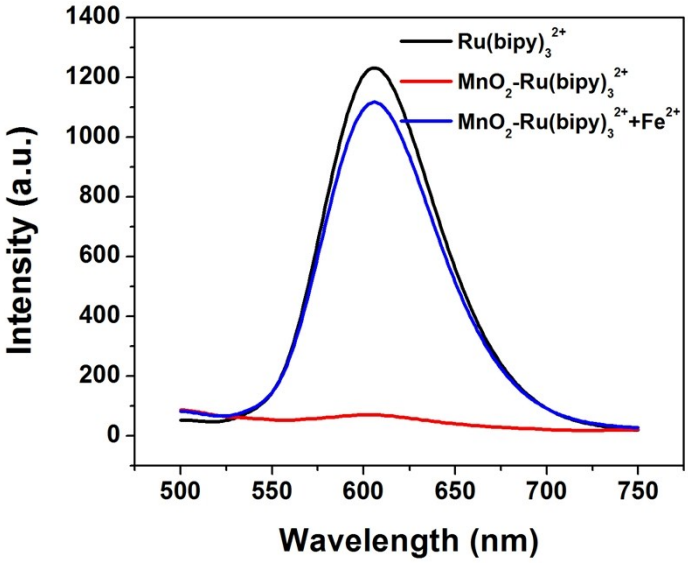

Fig. S5 Fluorescence emission spectra of $Ru(bipy)_3^{2+}$ and MnO_2-$Ru(bipy)_3^{2+}$ with and without Fe^{2+}.

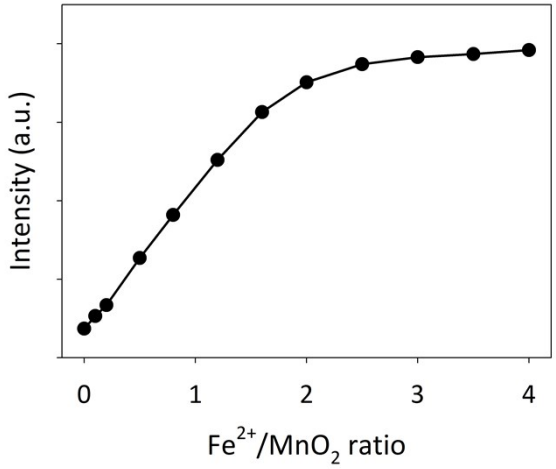

Fig. S6 Plot of fluorescence intensity at 594 nm against the Fe^{2+}/MnO_2 molar ratio.

During this redox reaction, Fe^{2+} was oxidized to generate Fe^{3+} through an intermolecular electron transfer as shown in eq 1.

$$MnO_2 + 2Fe^{2+} + 4H^+ \longrightarrow Mn^{2+} + 2Fe^{3+} + 2H_2O \qquad (1)$$

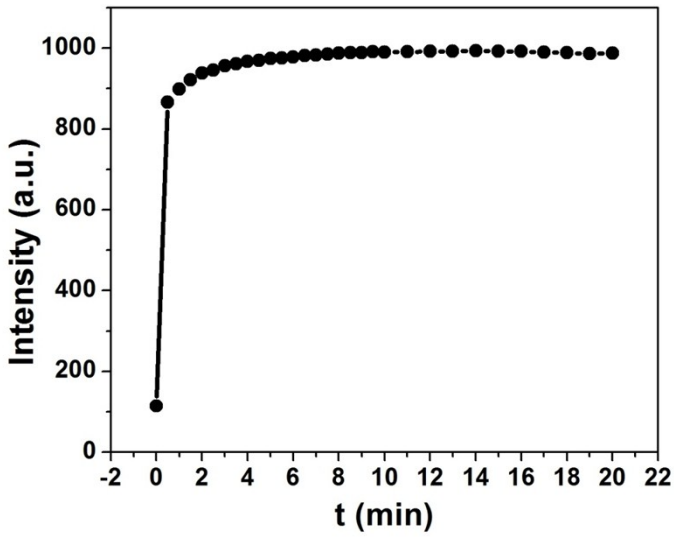

Fig. S7 Time dependence of fluorescence intensity of MnO_2-$Ru(bipy)_3^{2+}$ in the presence of Fe^{2+} (2 mM).

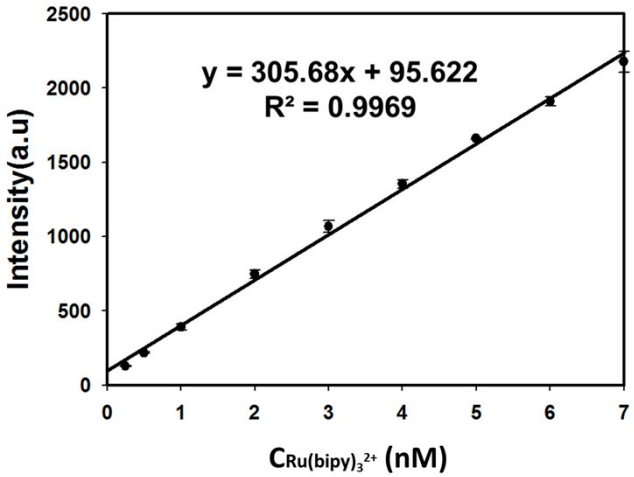

Fig. S8 Fluorescence emission intensity of $Ru(bipy)_3^{2+}$ solution with different concentrations.

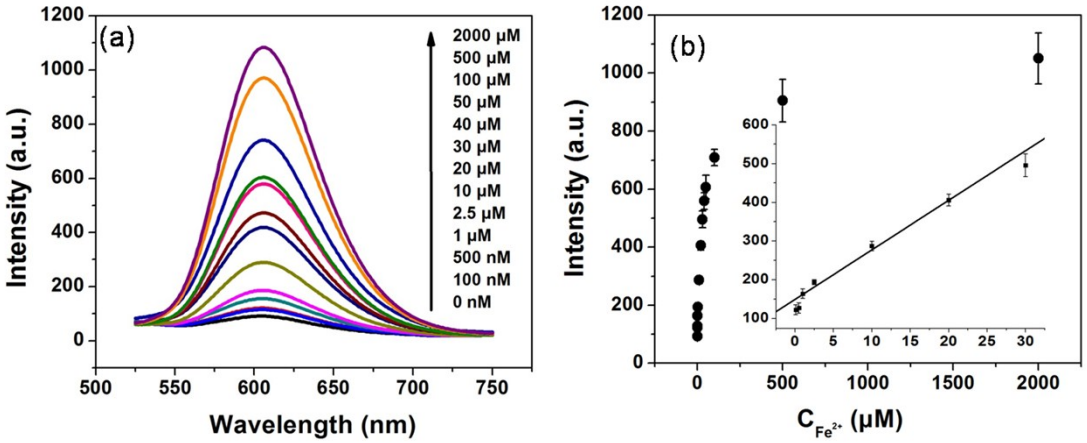

Fig. S9 (a) Fluorescence emission spectra of $Ru(bipy)_3^{2+}$ in the presence of MnO_2 (80 μg mL^{-1}) restored by different concentrations of Fe^{2+} (0-2000 μM). (b) The plot of the fluorescence intensity versus Fe^{2+} concentration.

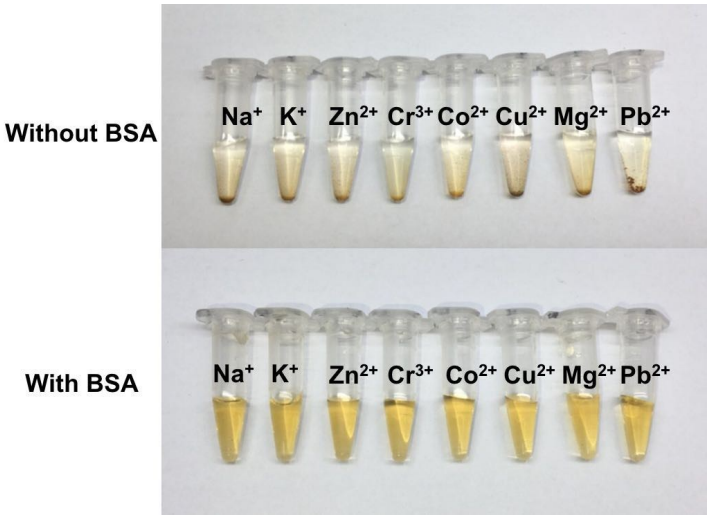

Fig. S10 The photo of MnO_2 nanosheets in the high concentration salt solution without or with BSA (100 μg mL^{-1}).

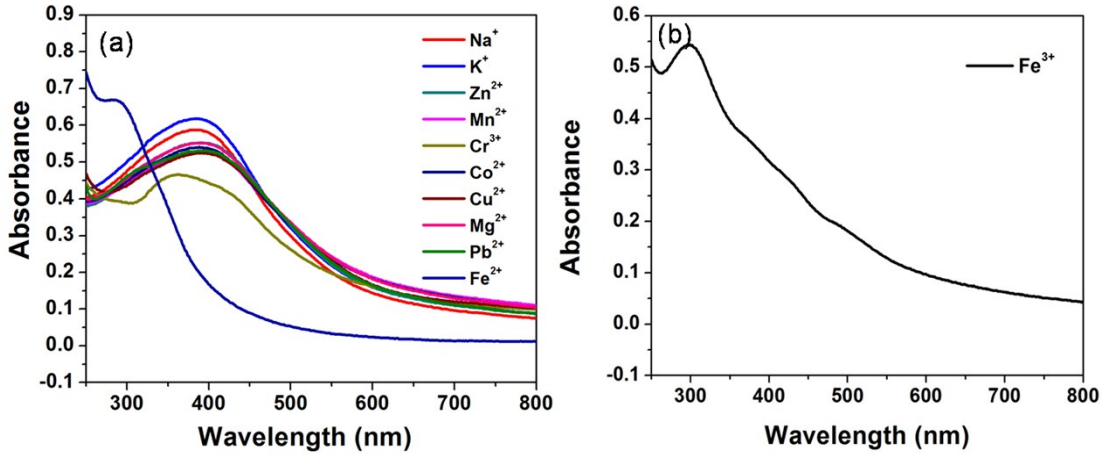

Fig. S11 (a) Absorption spectra of single-layer MnO_2 nanosheets after treatment with various metal ions. (b) Absorption spectrum of Fe^{3+}.

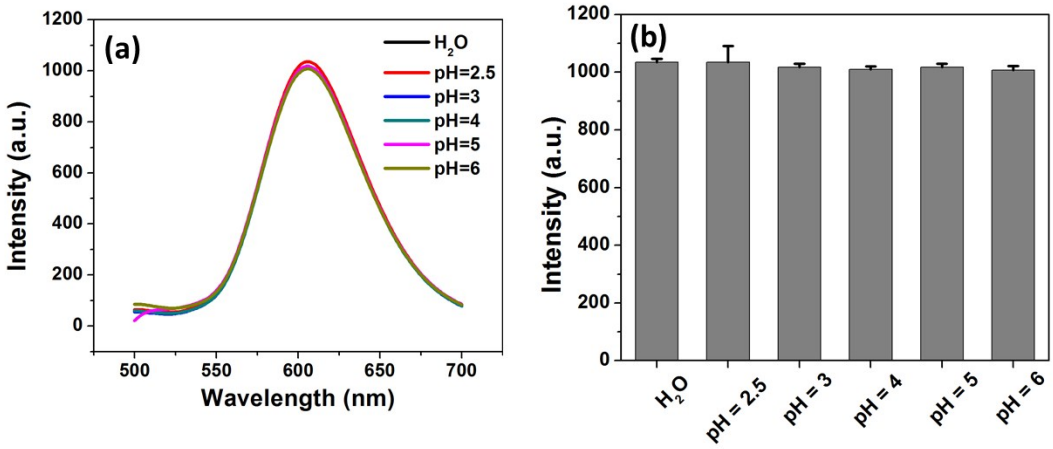

Fig. S12 Fluorescence emission spectra of $Ru(bipy)_3^{2+}$ in the presence of MnO_2 (80 μg mL^{-1}) treated with Fe^{2+} (500 μM) under different pH values condition.

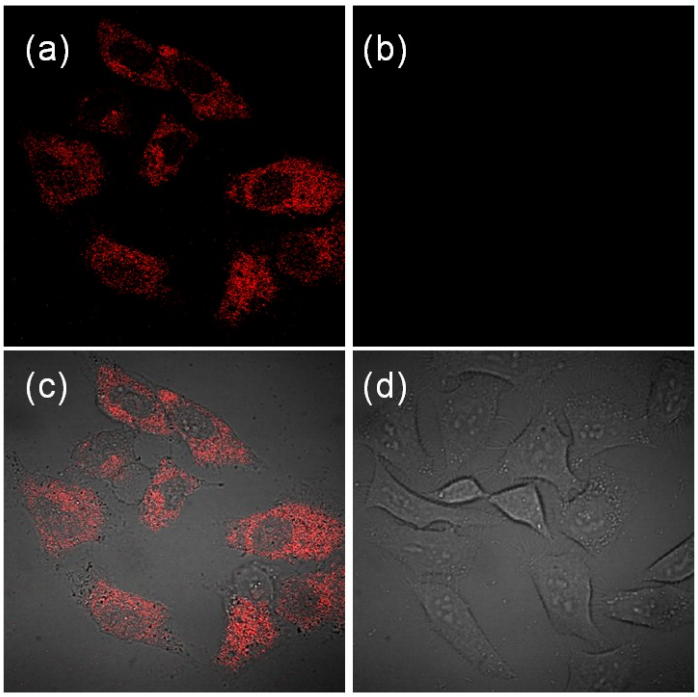

Fig. S13 CLSM images of HeLa cells incubated with MnO_2-$Ru(bipy)_3^{2+}$ (60 μg mL^{-1}) for 3 h (a, c), and HeLa cells pre-treated with NEM (500 μM) for 30 min and then incubated with MnO_2-$Ru(bipy)_3^{2+}$ (60 μg mL^{-1}) for 3 h (b, d).

Table S1. Comparison of different methods for Fe^{2+} sensing

probe	analytical methods	linear range	LOD	real sample	ref.
1,10-phenanthroline	sequential injection analysis	0.25 - 5.0 mg L^{-1}	18 μg L^{-1} (0.64 μM)	multi-vitamin tablets	2
2,2-bipyridyl	sequential injection analysis	5.0 - 40.0 mg L^{-1}	0.97 mg L^{-1} (34.6 μM)	ferrous sulfate syrup	3
PVC membrane based on NPA15C5	ion selective electrode	1.0×10^{-6} - 1.0×10^{-2} M	7.5×10^{-7} M	ferrous sulfate syrup	4
rhodamine-dimethyliminocinnamyl	electrochemical	potentiometric 1.0×10^{-1} - 1.0×10^{-7} M voltammetric 2.0×10^{-6} - 3.0×10^{-4} M	7.43×10^{-8} M 1.6×10^{-7} M	syntheticwater, ferrous sulphate tablets, ferrous syrup	5
polypyrrole and sodium dodecyl sulfate	electrochemical	potentiometric 1.0×10^{-6} -1.0×10^{-1} M voltammetric 1.0×10^{-9} - 1.0×10^{-5} M	8.78×10^{-7} M 5.8×10^{-10} M	ferrous fumarate tablets	6
ferrozine	spectrophotometric	10 - 80 μM	6 μM	in aqueous biological solutions	7
azo dye derivatives of pyrocatechol	spectrophotometric	no give	no give	multi-vitamin	8
silver nanoclusters	colorimetric	5 - 100 μM	76 nM	ferrous sulfate tablet	9
fluoran dye	colorimetric	no give	no give	no give	10
$[Ru(bpy)_2(Htppip)](ClO_4)_2\cdot H_2O\cdot DMF$	colorimetric	no give	4.46×10^{-8} M	no give	11
GSH-CdTe QDs	fluorescence	0.01 - 1 μM	5 nM	tap water and river water samples	12
MnO_2-$Ru(bipy)_3^{2+}$	fluorescence	0.1 - 15 μM	60 nM	ferrous sulfate tablet, living cells	**this work**

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