Electronic Supplementary Information for

Highly Selective Imaging of Lysosomal Azoreductase under Hypoxia Using pH-Regulated and Target-Activated Fluorescent Nanoprobes

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1. Experimental Section:

1.1 Reagents and materials.

All chemical reagents were purchased from Alfa Aesar. The azoreductase was purchased from CHI Scientific (Jiangsu China). Nicotinamide adenine dinucleotide phosphate (NADPH) was purchased from Beyotime (Shanghai, China). The dicoumarol was purchased from Sigma-Aldrich. The nigericin was purchased from Invitrogen (Thermo Fisher Scientific, USA). β-lapachone was purchased from J&K Scientific Ltd. All the chemicals were of analytical grade and used without further purification. Sartorius ultrapure water (18.2 MΩ cm) was used throughout the experiments. DNA oligonucleotides were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China) and purified by high-performance liquid chromatography (HPLC). The sequences of the oligonucleotides are described in Table S1.

1.2 Instruments.

Transmission electron microscopy images were obtained on JEM-100CXII microscope (JEOL, Ltd., Japan). UV-Vis absorption spectra were recorded on a Hitachi U-4100 UV/Vis spectrophotometer (Kyoto, Japan). Fluorescence images of cells were obtained using an Olympus FV1000-MPE multiphoton laser scanning confocal microscope (Japan).The pH was measured with a model 868 pH meter (Orion).

1.3 Synthesis of AuNPs.

$HAuCl_4$ (20 mL, 0.25 M) and trisodium citrate dihydrate (500 mL, 10 mM) were mixed with 19 mL H_2O in a round-bottom flask. Under vigorous stirring, 0.3 mL of freshly prepared NaBH4 solution (0.2 M) was quickly injected into the solution, leading to an immediate color change to yellowish red. After stirring for 1 h, the solution was maintained at 4 °C for 12-24 h and collected as the seed solution for subsequent seeded growth. Under vigorous stirring, a predetermined amount of the seed solution (4.7 mL) was then quickly injected into a freshly prepared growth solution containing 2 mL of PVP (10 wt%), 1 mL of L-ascorbic acid (0.2 M), 0.5 mL of KI (0.6 M), 0.3 mL of $HAuCl_4$ (0.5 M), and 20 mL of H_2O. After 10 minutes, the formed AuNPs were collected by centrifugation (two times) and redispersed in 40 mL water. The concentration of

AuNPs was determined to be 3.14 nM by UV-vis absorbance spectroscopy based on an extinction coefficient of 1.96 *109 M^{-1} cm^{-1} at l= 531 nm.

1.4 Preparation of βCD-AuNPs

For the surface modification process, 0.5 mg of SH-bCD was added to 500 mL AuNP colloidal solution. The mixture was then incubated for 24 h at room temperature. Then, the resulting mixture was subjected to centrifugation at 8500 rpm for 6 minutes, and the supernatant was removed, while the oil-like AuNPs precipitate was dissolved in ultrapure water to keep the final volume identical. These modified AuNPs solutions were stored at 4°C in a freezer.

To detect the βCD surface coverage of AuNPs, we mixed superfluous 200 nM P2 and 1 nM βCD-AuNPs for 24 h in order to make Azo enter into the cavity of βCD as much as possible. The resulting mixture was subjected to centrifugation 8500 rpm for 6 minutes, then, we pipetted 500 μL of the supernatant to detect the fluorescence intensity. At the same time, we detected the fluorescence intensity of different concentrations of Azo-P2-TAMRA to make calibration curve, then we used the calibration curve to calculate the concentration of Azo-P2-TAMRA that did not enter the cavity of βCD, so we can calculate the βCD surface coverage of AuNPs.

1.5 Preparation of nanoprobe.

The multilabeled P1 was added to the solution containing βCD–AuNPs and incubated to fabricate nanoprobe. Then, the resulting mixture was subjected to centrifugation at 8500 rpm for 6 minutes, and the supernatant was removed for the purpose of removing excess P1, while the oil-like precipitate was dissolved in ultrapure water to keep the final volume identical.

1.6 Cleavage of azobenzene moiety to release P1.

The constructed nanoprobe were incubated with azoreductase (100 μg/mL) and 50 μM NADPH at 37°C for 6 h under hypoxic condition (1% O_2). Then, the resulting mixture was subjected to centrifugation at the speed of 8500 rpm for 6 min and the substratum was removed with the purpose of removing excess βCD-AuNPs. The supernatant was collected. The fluorescence of TAMRA and Cy5 used the maximal excitation wavelength at 542 nm, and collected between 560 and 750 nm.

1.7 Cell culture.

HepG2 cells were cultured in DMEM medium supplemented with 10% fetal bovine serum and

100 U/mL 1% antibiotic penicillin / streptomycin and maintained at 37 °C in a 100% humidified atmosphere containing 5% CO_2.

1.8 MTT assay.

HepG2 cells (1×10^6 cells/well) were cultured with 100 μL of fresh culture medium (DMEM) in 96 well microtiter plates in a 5% CO_2/95% air incubator for 24 h. After the supernatant (the medium) was removed and washed three times with DMEM, the nanoprobe or β-lapachone was added to each well with different concentrations for 24 h. Then, 60 μL of MTT solutions (0.5 mg/mL) were added into each well and incubated together for another 4.0 h while the supernatant was following removed. Finally, we added DMSO (150 μL per well) to each well to dissolve the formazan crystals. The absorbance value was read at 490 nm with a RT 6000.

1.9 Imaging azoreductase in HepG2 cells under hypoxia.

The HepG2 cells were grown in DMEM cell medium with 10% inactivated fetal bovine serum at 37 °C in a humidified incubator containing 5% CO_2 (20% O_2), then incubated at 37°C atmosphere for 3 h under different conditions (20%, 10%, 5% and 1% O_2), followed by incubated with our nanoprobe (particle concentration, 0.5 nM) for another 1h, respectively. Before use, the adherent cells were washed with 1 mL of PBS buffer (pH 7.4) to remove the free nanoprobe. The nanoprobe-incubated cells were subjected to image by Olympus FV1000-MPE multiphoton laser scanning confocal microscope with exciting wavelengths at 561 nm for TAMRA and Cy5 channel (conditions: red fluorescence channel, λex = 546 nm and λem = 560-620 nm; green fluorescence channel, λex = 546 nm and λem > 620 nm).

1.10 Imaging azoreductase in HepG2 cells treat with nigericin under hypoxia.

The HepG2 cells were grown in DMEM cell medium with 10% inactivated fetal bovine serum at 37 °C in a humidified incubator containing 5% CO_2 (20% O_2). After removing the medium, cells were incubated at 37°C atmosphere for 3 h under hypoxia conditions (1% O_2) in fresh medium, followed by incubated with our nanoprobe (particle concentration, 0.5 nM) for another 1h, and before imaging, cells were washed three times by D-PBS and treated with high K^+ HEPES-buffered solution (20 mM NaCl, 125 mM KCl, 0.5 mM $CaCl_2$, 0.5 mM $MgCl_2$, 5 mM glucose, and 20 mM HEPES) at various pH values (pH 5.0, 5.5, 6.0, 6.5, and 7.0) in the presence of 10.0 μM nigericin. After 15 min, the nanoprobe-incubated cells were subjected to image by Olympus

FV1000-MPE multiphoton laser scanning confocal microscope with exciting wavelengths at 561 nm for TAMRA and Cy5 channel (conditions: red fluorescence channel, λex = 546 nm and λem = 560-620 nm; green fluorescence channel, λex = 546 nm and λem > 620 nm). The fluorescence images and pseudoimages were presented after processing by software Image ProPlus 6.0

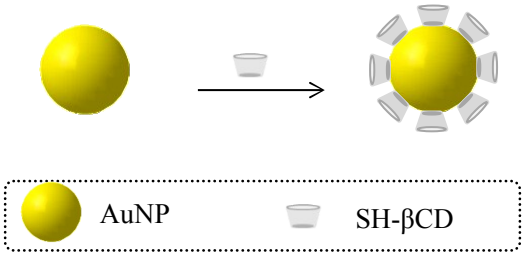
1.11 Imaging lysosomal azoreductase with β-lapachone treatment under hypoxia.

The HepG2 cells were grown in DMEM cell medium with 10% inactivated fetal bovine serum at 37 °C in a humidified incubator containing 5% CO_2 (20% O_2). After removing the medium, cells incubated with nanoprobe (particle concentration, 0.5 nM) for 1h, after that the medium containing contain nanoprobe was discarded and FBS free medium with different concentrations were incubated with cells under mild hypoxia (~10% O_2) for 3h. Before use, the adherent cells were washed with 1 mL of PBS buffer (pH 7.4) to remove the free nanoprobe. The HepG2 cells were subjected to image by Olympus FV1000-MPE multiphoton laser scanning confocal microscope with exiting wavelengths at 561 nm for TAMRA and Cy5 channel (conditions: red fluorescence channel, λex = 546 nm and λem = 560-620 nm; green fluorescence channel, λex = 546 nm and λem > 620 nm).

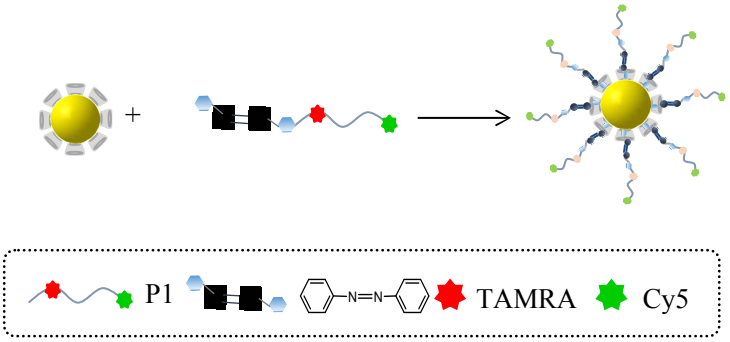
1.12 Detecting ROS generation with β-lapachone treatment under mild hypoxia.

The HepG2 cells were grown in DMEM cell medium with 10% inactivated fetal bovine serum at 37 °C in a humidified incubator containing 5% CO_2 (20% O_2). Different concentrations of β-lapachone were incubated with HepG2 cells for 3h under hypoxia and then fixed with 4% paraformaldehyde for 20 min followed by adding SOSG working solution (10μM) and incubated for 30 min. Then, the cell medium was removed and the cells were washed with PBS to image by Olympus FV1000-MPE multiphoton laser scanning confocal microscope with exiting wavelengths at 488nm and collecting at 510-550nm.

1. The Schematic Synthesis of The βCDs-AuNPs and Nanoprobe



Scheme S1. The schematic synthesis of βCDs-AuNP.



Scheme S2. The schematic synthesis of nanoprobe.

3. Oligonucleotides Sequences

Table S1. Oligonucleotides Sequences Used in This Work[1]

Name	Sequence (5 '--3 ')
P1	5′-Cy5-TATCCCTAACCCTAACCCTAACCCATA-TAMRA-TTTTT (Azo) TTTTT -3′
P2	5′- TAMRA-CTAACACTAACACTAAC-TTTTT (Azo) TTTTT-3′

4. Experimental Data

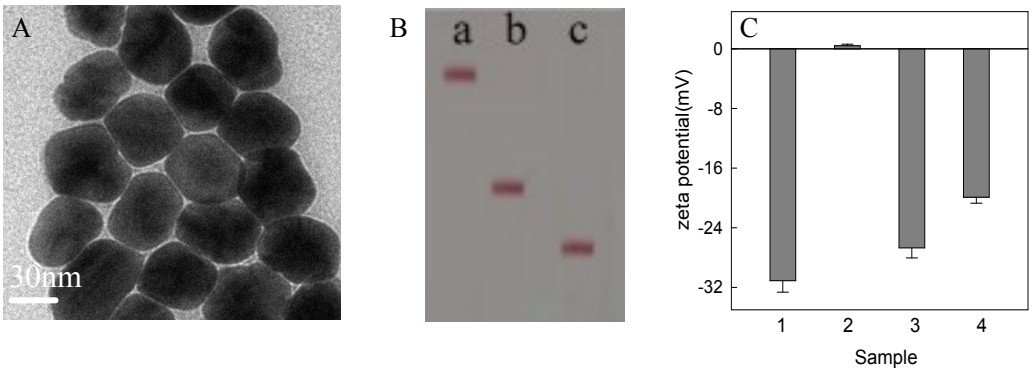


Fig.S1 (A) TEM images of our constructed nanoprobe. (B) 2% agarose gel electrophoresis image of AuNPs (lane a), βCD-AuNPs (lane b), and the formed nanoprobe (lane c). (C) Zeta petential of AuNPs(1); βCD-AuNPs (2); nanoprobe(3); and nanoprobe @CPP (4).

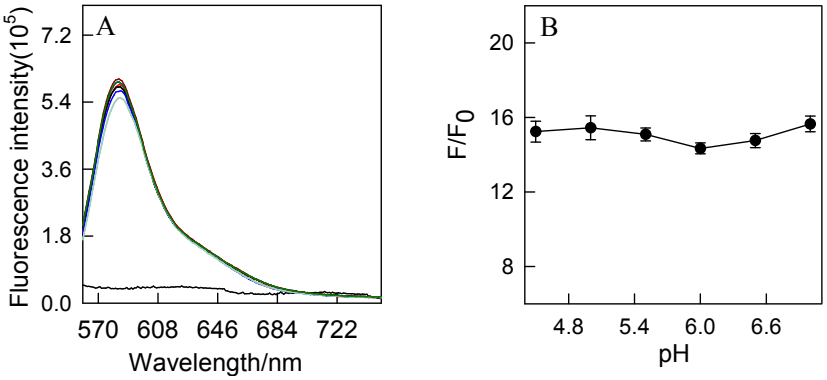


Fig. S2 (A) Fluorescence emission spectra of βCD-AuNPs-P2 under different conditions: The βCD-AuNPs-P2 in PBS buffer (pH 7.0; black curve); the βCD-AuNPs-P2 in the presence of azoreductase (75 μg/mL) and NADPH (50 μM) under hypoxia conditions in buffer at pH 5.0-7.4. (B) Fluorescence enhancement (F/F_0, where F and F_0 represents the fluorescence intensity of TAMRA) as functions of pH upon azoreductase (75 μg/mL) and 50μM NADPH addition under hypoxia (1% O_2).

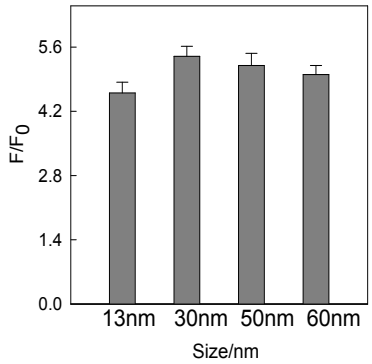


Fig. S3 Fluorescence enhancement (F/F_0, where F and F_0 represent the fluorescence intensity of TAMRA after and before azoreductase (75 μg/mL) and NADPH (50 μM) addition of the nanoprobe as functions of different sizes of AuNPs at pH 5.0 under hypoxia condition (1% O_2). Error bars represent variations between three measurements.

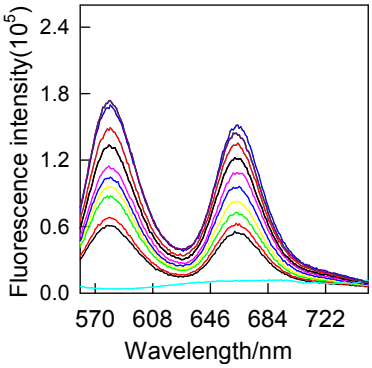


Fig. S4 Fluorescence emission spectra of nanoprobe reacted with different concentrations (0, 15, 30, 45, 60, 75, 90, 105 μg/mL, pH 5.0) of azoreductase and NADPH (50 μM) at 37 °C for 6 h under hypoxic condition (1% O_2).

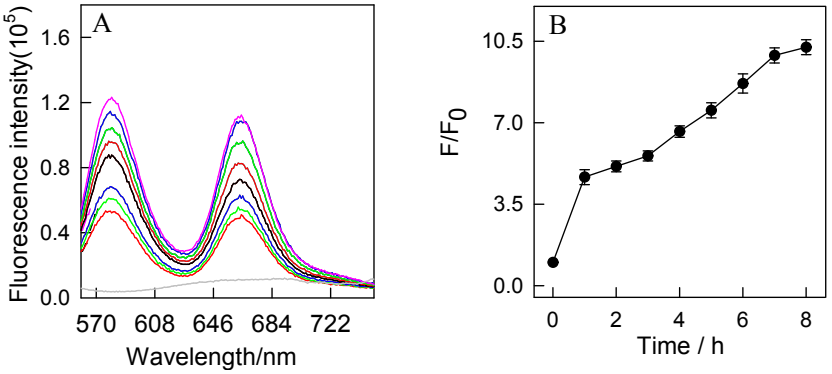


Fig. S5 (A) Fluorescence emission spectra of nanoprobe with varied reaction time (1, 2, 3, 4, 5, 6, 7, 8 h, pH 5.0) in the presence of azoreductase (75 μg/mL) and NADPH (50 μM) under hypoxia condition (1% O_2). (B) Calibration curve of fluorescence signal enhancement (F/F_0, where F and F_0 represent the fluorescence intensity of Cy5 after and before azoreductase (75 μg/mL) and NADPH (50 μM) addition with varied reaction time (1, 2, 3, 4, 5, 6, 7, 8 h, pH 5.0) under hypoxia condition (1% O_2). Error bars represent variations between three measurements.

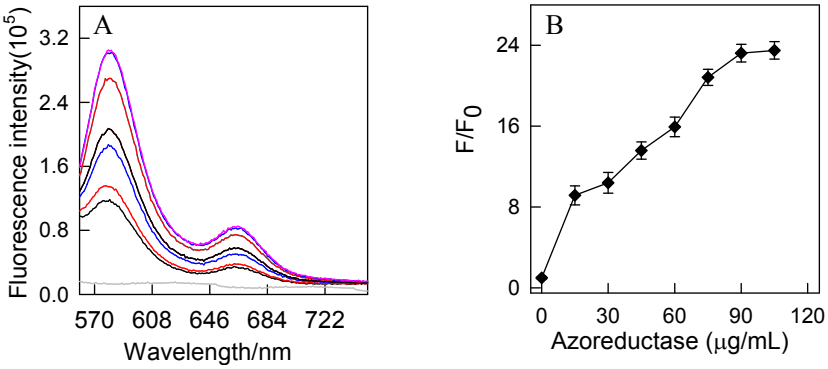


Fig. S6 (A) Fluorescence emission spectra of nanoprobe reacted with different concentrations (0, 15, 30, 45, 60, 75, 90, 105 μg/mL, pH 7.4) of azoreductase and NADPH (50 μM) at 37 ℃ for 6 h under hypoxic condition (1% O_2). (B) Calibration curve of fluorescence signal enhancement (F/F_0, where F and F_0 represents the fluorescence intensity of TAMRA after and before addition with varied concentration: 0, 15, 30, 45, 60, 75, 90, 105 μg/mL, pH 7.4) under hypoxia condition (1% O_2). Error bars represent variations between three measurements.

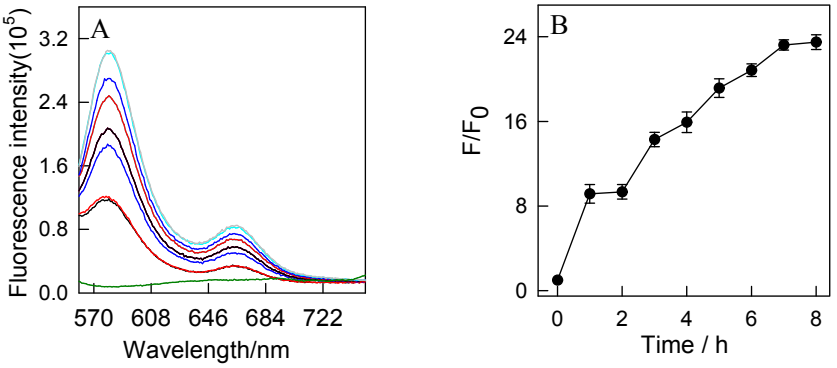


Fig. S7 (A) Fluorescence emission spectra of nanoprobe with varied reaction time (1, 2, 3, 4, 5, 6, 7, 8 h, pH 7.4) in the presence of azoreductase (75 μg/mL) and NADPH (50 μM) under hypoxia condition (1% O_2). (B) Calibration curve of fluorescence signal enhancement (F/F_0, where F and F_0 represents the fluorescence intensity of Cy5 after and before azoreductase (75 μg/mL) addition with varied reaction time (1, 2, 3, 4, 5, 6, 7, 8 h, pH 7.4) under hypoxia condition (1% O_2). Error bars represent variations between three measurements.

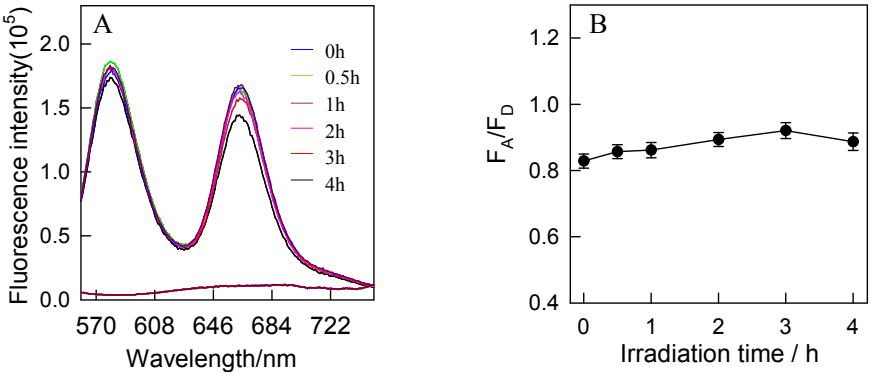


Fig. S8 (A) Fluorescence emission spectra of nanoprobe under different conditions: nanoprobe with varied irradiation time (0, 0.5, 1, 2, 3, 4h, bottom lines); nanoprobe in the presence of azoreductase (100μg/mL) and NADPH (50μM) at 37 °C for 6 h under hypoxia condition (1% O_2, pH 5.0) with varied irradiation time (0, 0.5, 1, 2, 3, 4h, top lines).The excitation wavelength was 542nm. (B) Plot of F_A/ F_D as a function of irradiation time (F_A, F_D represent the fluorescence intensity of TAMRA and Cy5 upon azoreductase (100μg/mL) and NADPH (50μM) addition). Error bars represent variations between three measurements.

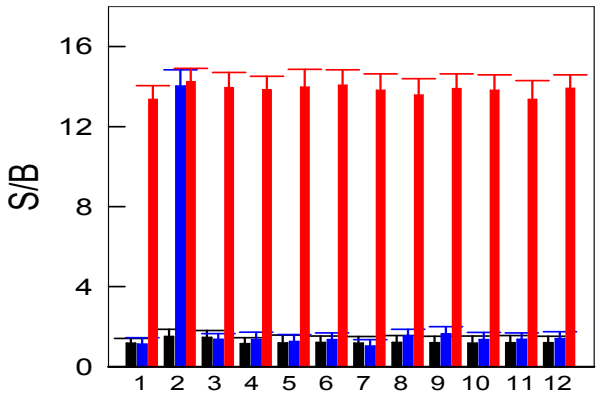


Fig. S9 S/B of nanoprobe (0.5nM) reacted with different kinds of species, blank(1), NADPH (50 μM) (2), Cyt a (1 mM) (3), tyrosine (1 mM) (4), glycine (1 mM) (5), GSH (1 mM) (6), vitamin C (1 mM) (7), H_2O_2 (1 mM) (8), glucose (10 mM) (9), and BSA (10 mg/mL) (10), $FeCl_2$ (1mM) (11), NaClO (10mM) (12). Black bars represent the cases in absense of azoreductase with NADPH (50 μM) under hypoxia condition (1% O_2, pH 5.0). Blue bars represent the cases in the presence of azoreductase (75 μg/mL) without NADPH (50 μM) under hypoxia condition (1% O_2, pH 5.0). Red bars represent the cases in the presence of azoreductase (75 μg/mL) with NADPH (50 μM) under hypoxia condition (1% O_2, pH 5.0). S/B was calculated from Cy5 fluorescence intensity. Error bars represent variations between three measurements.

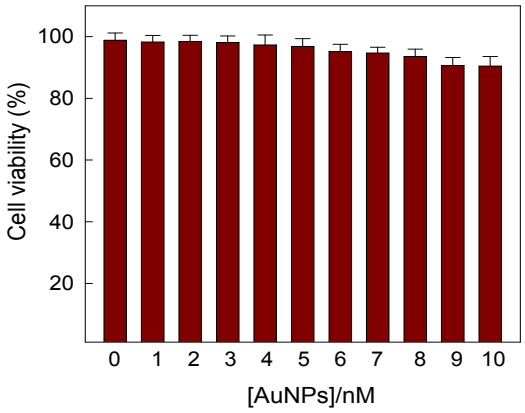


Fig. S10 Cell relative viability of HepG2 cells after treated with the different concentration of nanoprobe: 0-10 represents the concentration of nanoprobe: 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1 nM, respectively. The concentration of nanoprobe was defined by AuNPs. Error bars represent variations between three measurements.

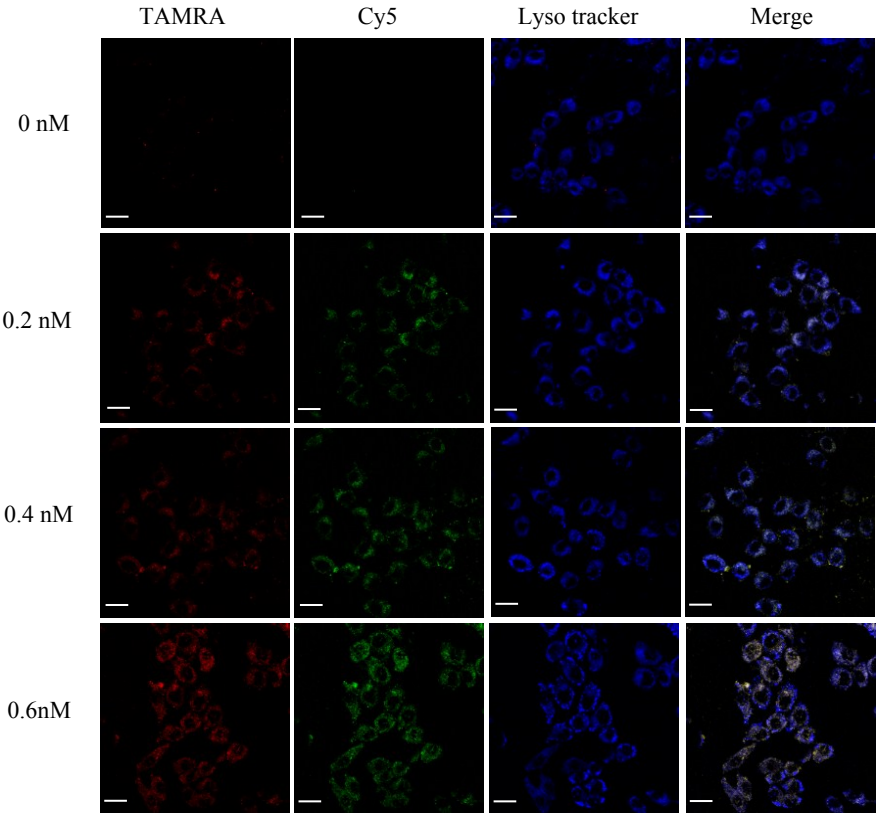


Fig. S11 Confocal fluorescence images of HepG2 cells under hypoxia condition (1% O_2) incubated with nanoprobe for different concentrations (0, 0.20, 0.40, 0.60 nM) at 37°C for 1 h, following incubated at 37 °C atmosphere for 3 h. (Red color: TAMRA, green color: Cy5, blue color: lysosome stained by Lyso Tracker Blue.) Scale bar: 20 μm. The concentration was defined by AuNPs.

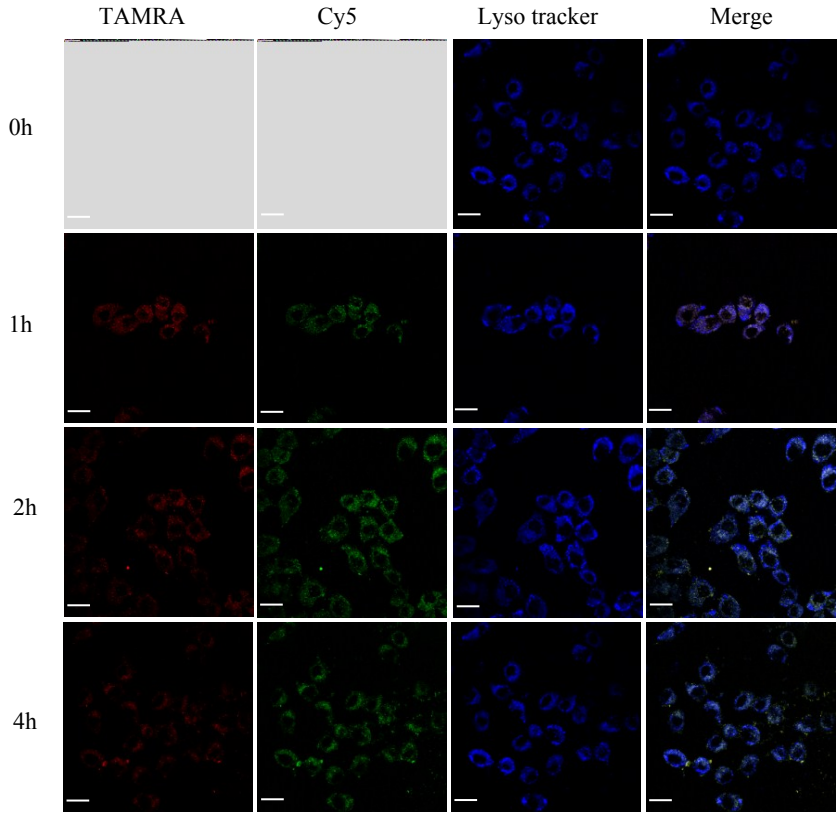


Fig. S12 Confocal fluorescence images of HepG2 cells under hypoxia condition (1% O_2) incubated with nanoprobe (0.5nM) for different times (0h, 1h, 2h, 4h) at 37°C for 1 h, following incubated at 37 °C atmosphere for 3 h. (Red color: TAMRA, green color: Cy5, blue color: lysosome stained by Lyso Tracker Blue.) Scale bar: 20 μm. The concentration was defined by AuNPs.

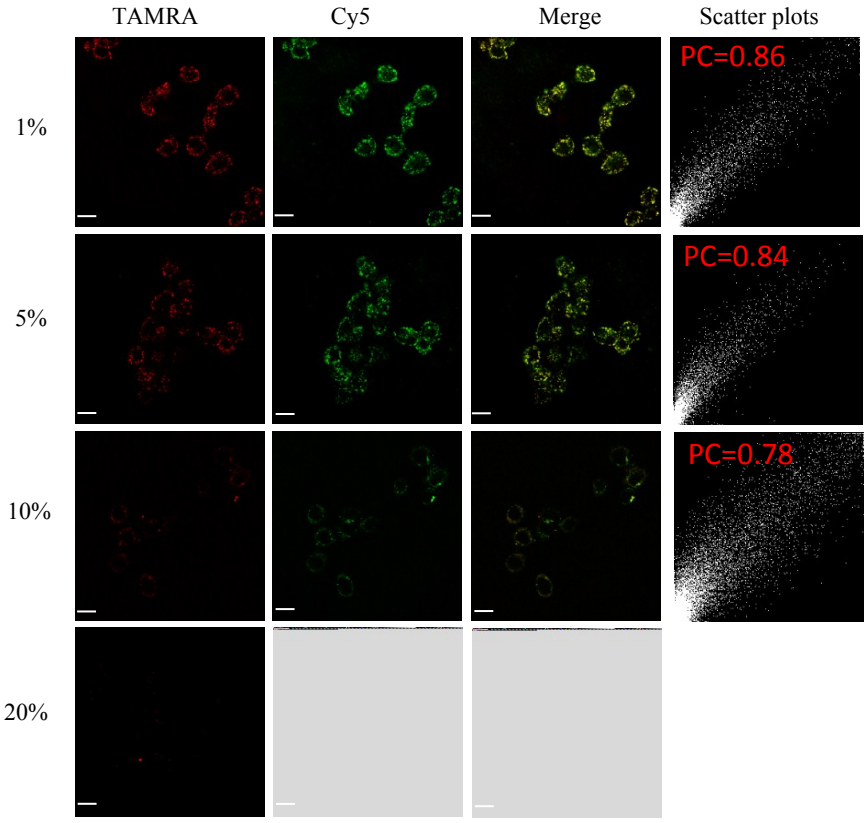


Fig. S13 Confocal fluorescence images of HepG2 cells under normoxic (20% O_2) and different hypoxia conditions (10%, 5% and 1% O_2) incubated with our nanoprobe (0.5nM) at 37°C for 1 h and then incubated at 37°C atmosphere for 3 h. (Red color: TAMRA, green color: Cy5.) Scale bar: 20 μm. The concentration was defined by AuNPs.

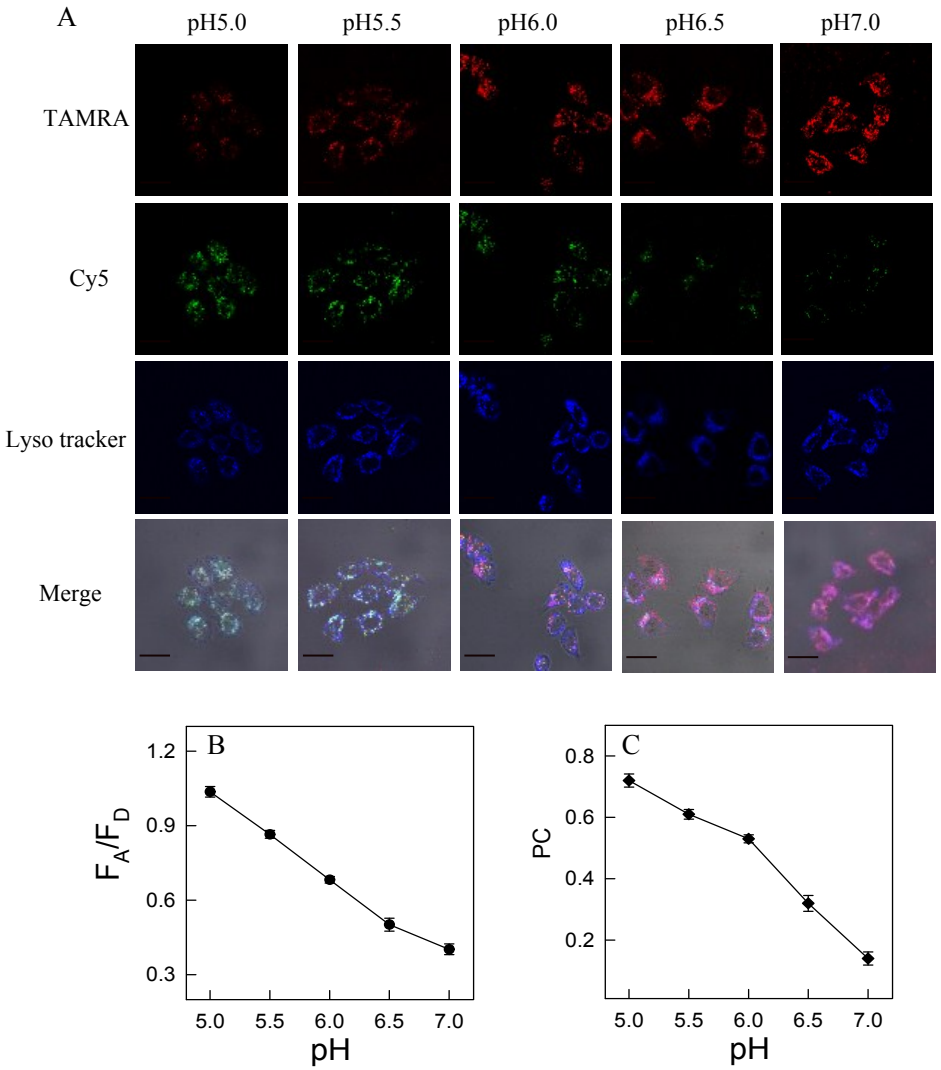


Fig. S14 (A) Confocal fluorescence images of HepG2 cells incubated at 37°C atmosphere for 3 h followed by incubated with our nanoprobe for another 1 h under hypoxia conditions (1% O_2), then exposed to external media at various pH (5.0, 5.5, 6.0, 6.5 and 7.0, respectively) in the presence of 10.0 μM nigericin. The excitation wavelength was 546 nm, and the images were collected in the ranges of 560-620nm (TAMRA-red) and 625-675nm (Cy5-green). (Blue color: lysosome stained by Lyso Tracker Blue). Scale bar: 20 μm. (B) Plot of A/D as a function of pH in a cell system. (C) Linear curve with decreasing Pearson's coefficient (PC, indicated localization effect of Lyso Tracker Blue and Cy5) as a function of pH. Error bars represent variations between three measurements.

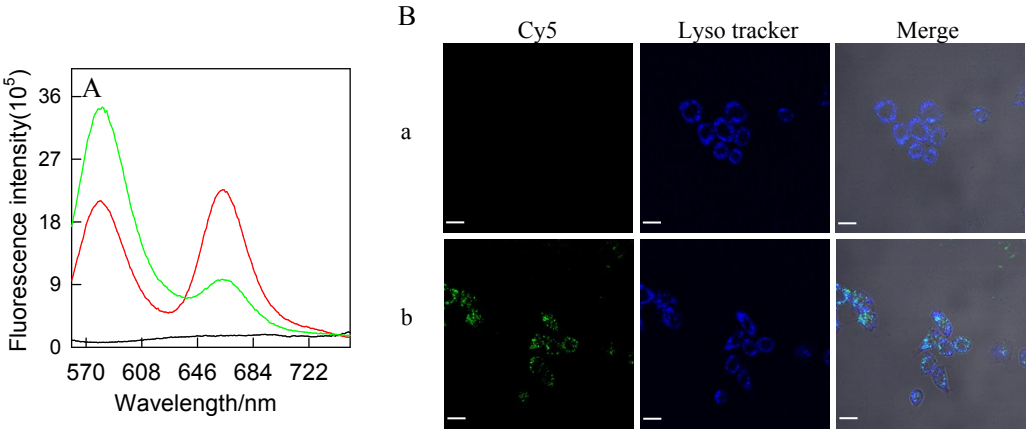


Fig. S15 (A) Fluorescence emission spectra of nanoprobe@CPP under different conditions: The nanoprobe@CPP in PBS buffer (pH 7.0; black curve); the nanoprobe@CPP in the presence of azoreductase (75 μg/mL) and NADPH (50 μM) under hypoxia conditions at pH 5.0 (red curve) and 7.4 (green curve). (B) Control experiments in HepG2 cells under hypoxia condition (1% O_2) incubated with nanoprobe@CPPs (0.5nM) for 1 h and then incubated at 37°C atmosphere for 3 h (a); then treated with nigericin and exposed to external media at pH 5.0 (b), respectively. (Green color: Cy5, blue color: lysosome stained by Lyso Tracker Blue.) Scale bar: 20 μm. The concentration was defined by AuNPs.

Supplementary explanation: Cell penetrating peptides (CPPs, $CAAAAAAK^{+}(Me)_3$) were employed to functionalize our designed nanoprobe via the Au-S bond, which could transport the nanoparticles into the cytoplasma membrane in an energy-independent way and thus avoided endocytose them into the lysosomes.[2] The result in Fig. S15A demonstrated the conjugated CPP did not affect the interaction of azoreductase with nanoprobe.[3] Under imaging of laser confocal, we could not observe obvious Cy5 fluorescence after the HepG2 cells were incubated with nanoprobe@CPP followed by hypoxia (1% O_2) for 3 h. However, when these HepG2 cells were treated with new cell culture medium containing 10 μM nigericin at pH 5.0, we could observe bright fluorescence of Cy5 under excitation of TAMRA (Fig. S15B).

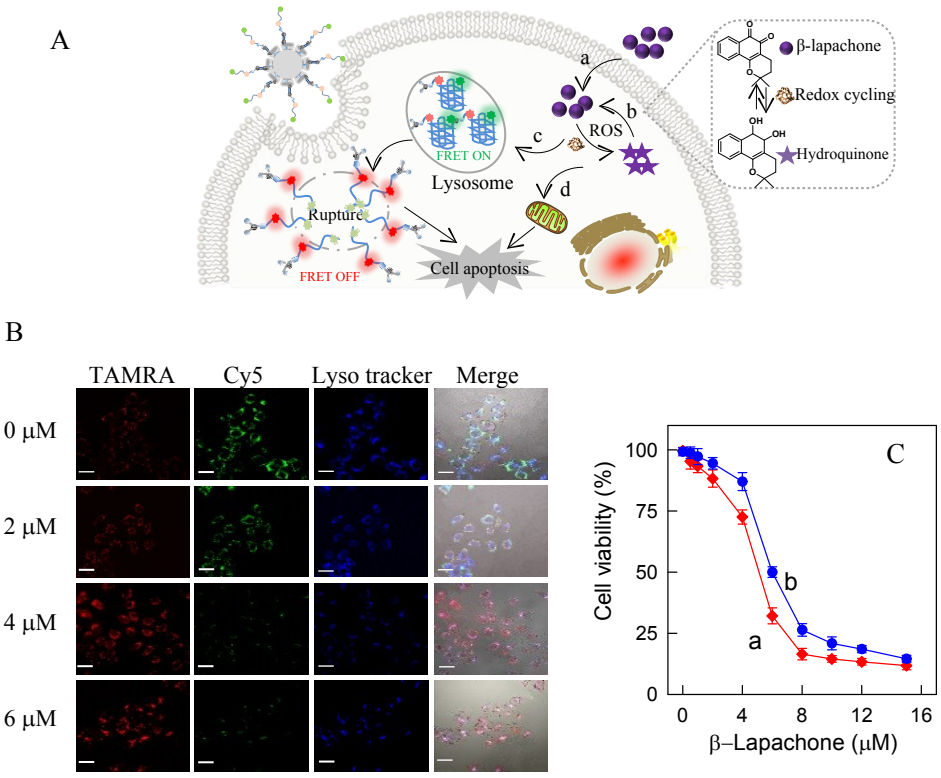


Fig. S16 (A) Schematic mechanism of the azoreductase activated cytotoxicity of β-lapachone under mild hypoxia (~10% O_2). (a. drug endocytosis b. redox cycling of drug c. ROS induce lysosome rupture d. ROS damage mitochondria, finally cell apoptosis). (B) Confocal fluorescence images of nanoprobe incubated-HepG2 cells upon treated with different concentrations of β-lapachone under mild hypoxia (~10% O_2). Scale bar: 20 μm. (C) Cell viability of HepG2 cells upon treated with the different concentrations of β-lapachone: 0, 0.5, 2, 4, 6, 8, 10, 12, 15μM under mild hypoxia (~10% O_2) (a) / nomorxic condition (20% O_2) (b).Error bars represent variations between three measurements.

Supplementary explanation: According to a previous report, azoreductase can activate the cytotoxicity of quinone-containing chemotherapeutic drugs through redox cycling pathways and can generate excessive ROS. To better understand biological function of azoreductases in this physiological process (Fig. S16A), β-lapachone was employed in our design. [4,5] As illustrated in Fig. S16B and S17, upon β-lapachone treatment under mild hypoxia (~10% O_2), the fluorescence intensity of TAMRA increased continually while the Cy5 weakened. This is mainly attributed to the β-lapachone treatment-generated redundant ROS could cause cell apoptosis through induce lysosome rupture (Figs. S18-S19). In this case, the released C-quadruplex structure P1 entered to cytoplasm demonstrated random coil (open state) with low FRET efficiency. The fluorescence intensity collected from Lyso Tracker Blue channel in Fig. 4B decreased with the increasing

concentration of β-lapachone further confirmed this point. Then, we subsequently studied the cytotoxicity of β-lapachone under hypoxia (~10% O_2, with positive expression of azoreductase), and found the IC_{50} (half-maximal inhibitory concentration of a substance) of β-lapachone under mild hypoxia was 4.91μM, which was lower than that under nomorxic (IC_{50} = 6.58μM) (Fig. S16C), this result ascribed to the high cytotoxic of β-lapachone catalysed by azoreductase under mild hypoxia. Taken together, these results confirmed the connection of azoreductase to certain quinone-containing chemotherapeutic drugs under hypoxia.

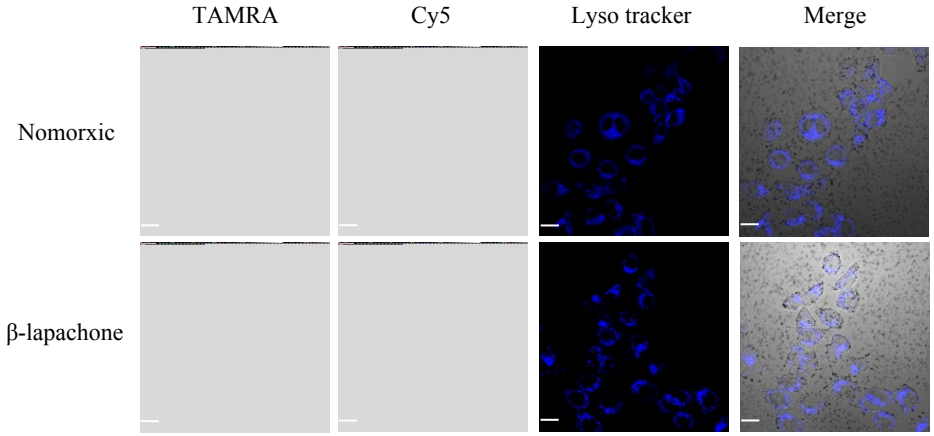


Fig. S17 Control experiments in HepG2 cells incubated with our nanoprobe (0.5nM) for 1 h and then incubated under nomorxic condition (20% O_2) and β-lapachone-only (4μM) under nomorxic condition (20% O_2) for 3 h. (Red color: TAMRA, green color: Cy5, blue color: lysosome stained by Lyso Tracker Blue.) Scale bar: 20 μm. The concentration was defined by AuNPs.

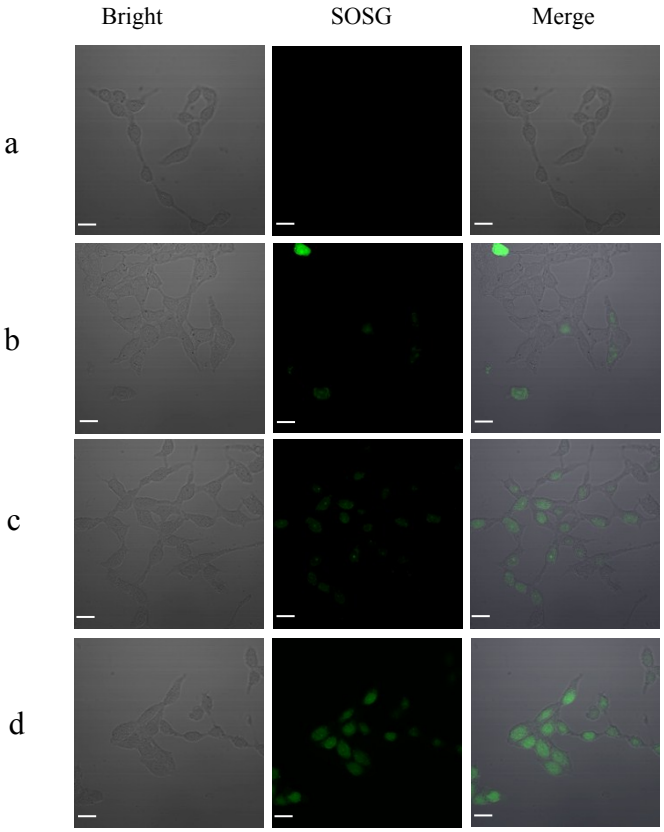


Fig. S18 Confocal fluorescence images of SOSG stained HepG2 cells with different treatments: control (a); β-lapachone-only (4μM) (b); mild hypoxia (~10% O_2) (c); β-lapachone (4μM) incubated with cells under mild hypoxia (~10% O_2) (d). Scale bar: 20 μm.

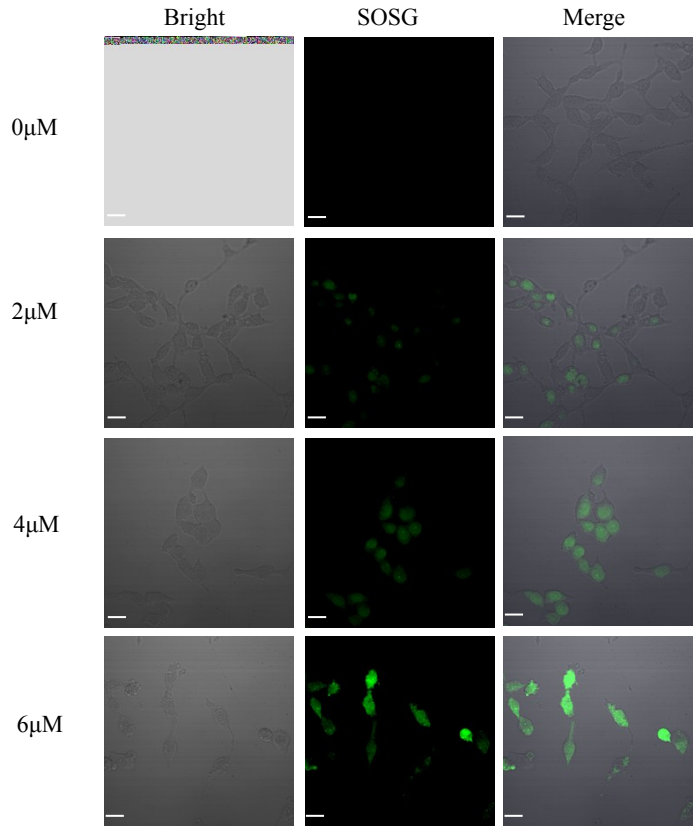


Fig. S19 Confocal fluorescence images of SOSG stained HepG2 cells with different concentrations of β-lapachone (0, 2, 4, 6μM) treatment under mild hypoxia (~10% O_2). Scale bar: 20 μm.

References:

1 J. Huang, L. Ying, X. Yang, Y. Yang, K. Quan, H. Wang, N. Xie, M. Ou, Q. Zhou and K. Wang, *Anal. Chem.*, 2015, **87**, 8724-8731.

2 S. Koutsopoulos, L. Kaiser, H. M. Eriksson and S. Zhang, *Chem. Soc. Rev.*, 2012, **41**, 1721-1728.

3 J. Tang, C. Huang, J. Shu, J. Zheng, D. Ma, J. Li and R. Yang, *Anal. Chem.*, 2018, **90**, 5865-5872.

4 L. Song, V. H. B. Ho, C. Chen, Z. Yang, D. Liu, R. Chen and D. Zhou, *Advanced Healthcare Materials*, 2013, **2**, 275-280

5 E. I. Parkinson and P. J. Hergenrother, *Acc. Chem. Res.*, 2015, **48**, 2715-2723.