Supporting Information for:

Hypoxic-triggered Gene Therapy: An New Drug Delivery System to Utilize the Photodynamic-induced Hypoxia for Synergistic Cancer Therapy

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1. Experimental Section:

1.1 Instruments

Ultrapure water (18.2 MΩ) obtained from a Milli-Q system was used through-out the study. All other chemicals were of analytical reagent grade and were used as received, unless otherwise stated. HepG2 cells were obtained from the American Type Culture Collection (Manassas, VA).

1.2 Flow Cytometry Experiments

HepG2 cells and L02 cells incubated with FITC labelled-AS1411 aptamer-fabricated DDS 2 h. Then collected HepG2 cells and L02 cells, washed with PBS buffer three times and followed by flow cytometry analysis using a FACScan cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA).

1.3 Detecting singlet oxygen generation

To detect the singlet oxygen generation, singlet oxygen sensor green (SOSG) was introduced into a DDS@TMPyP4 and TMPyP4 solution, followed by irradiation using 660 nm laser (1 W cm^{-2}) for different periods of time. The SOSG fluorescence was obtained with excitation at 494 nm and maximum emission at 534 nm after irradiation to determine the sample's SOSG.

1.4 Intracellular singlet oxygen detection

HepG2 cells were seeded in confocal dishes and incubated overnight at 37 °C under a humidified 5% CO2 atmosphere. DDS@TMPyP4 was incubated with HepG2 cells for 1 hour and fixed with 4% paraformaldehyde for 20 min followed by adding 10 μL of SOSG working solution and incubating for 30 min. Then, the cells were irradiated for 20 min (660 nm, 1 W cm^{-2}).

Subsequently, the incubation medium was removed and the cells were washed with PBS and incubated with Hoechst 33342. Confocal images were collected with an Olympus FV1000-MPE multiphoton laser scanning confocal microscope.

1.5 In Vitro Photodynamic Therapy

For PDT, HepG2 cells were seeded in 96-well plate. Then, DDS@TMPyP4, control DDS-A@TMPyP4 and control DDS-B@TMPyP4 at various concentrations was added to the plates and incubated with cells for 1h. Then, the plates were placed in a lucifugal box, following by mild hypoxia (oxygen concentration ~10%) in advance for 1 h, and then irradiated with 660 nm laser at a power density of 1 W cm^{-2} for 30 minutes. After that, cells were placed in a cell culture box and further incubated for 48 h. The quantitative evaluation of the photodynamic cytotoxicity was performed by MTT assay as described above.

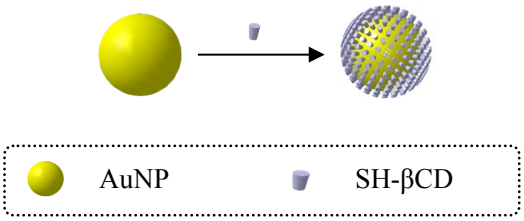
1.6 Western Immunoblotting

HepG2 cells (2×10^6 cells) were plated into 60 mm cell culture dishes in Dulbecco's modified eagle medium were seeded in culture dish and incubated overnight at 37 °C under a humidified 5% CO_2 atmosphere. The next day, the cells were pretreated with DDS@TMPyP4 and DDS-A@TMPyP4 incubated with cells for 1 h. Then, the plates were placed in a lucifugal box, following by mild hypoxia (oxygen concentration ~10%) for 1 h, and then irradiated with 660 nm laser at a power density of 1 W cm^{-2} for different time. After that, cells were placed in a cell culture box and further incubated for 48 h. The cells were extracted and washed three times with ice-cold PBS, centrifuged at 1000 rpm for 4 minutes, and the supernatant was aspirated. Add 60 uL of RIPA Lysis buffer to the cells and mix by pipetting repeatedly and lysed on ice for 10 minutes, then

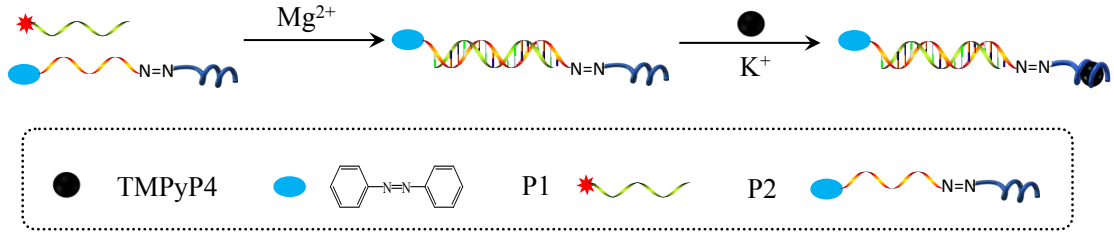
centrifuged at 12,000 rpm for 15 minutes at 4 °C. Collected the centrifuged supernatant and stored at -20 °C for experiments. Cell extracts were separated by SDS-PAGE and transferred to a nitrocellulose membrane. The membranes were blocked with 5% skim milk in Tris-buffered saline (TBS) containing 0.1% Tween-20 for 30 minutes at room temperature. After blocking, membranes were incubated with HIF-1α antibody overnight at 4 °C, followed by incubation with HRP-labeled Goat Anti-mouse-IgG for 2 h at room temperature. The membrane incubated with ECL chemiluminescence liquid (Thermo) for 3 minutes. Absorb the liquid with absorbent paper. Wrap the membrane with a cling film and expose the film to the X film within seconds to several minutes.

2. The schematic synthesis of βCDs-AuNP and DRHC-TAMRA@TMPyP4

Scheme S1. The schematic synthesis of βCDs-AuNP.



Scheme S2. The schematic synthesis of DRHC-TAMRA@TMPyP4 .



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3. Oligonucleotides Sequences

Table S1. Oligonucleotides Sequences Used in This Work

Name	Sequence (5 '--3 ')
sensing sequence	UCACCAAAGUUGAAUCAGA-TAMRA
antisensing sequence	UCUGAUUCAACUUUGGUGA
P1	UCACCAAAGUUGAAUCAGA-TAMRA
P2	T/Azo/TTTUCUGAUUCAACUUUGGUGATT/Azo/GAGACTTTGGTGGTGGTGGTGG TTGTGGTGGTGG
P3	T/Azo/TTTUCUGAUUCAACUUUGGUGATT/Azo/GAGACTTTGGTGGTGGTGGTGG TTGTGGTGGTGG-FITC
P4	T/Azo/TTTUCUGAUUCAACUUUGGUGATT/Azo/GAGACTTTCCGTGCCTGTGGCT GACCGTCGCATT-FITC
P5	GUACCGACGUGCAUUGACA-TAMRA
P6	T/Azo/TTTUGUCAAUGCACGUCGGUACTT/Azo/GAGACTTTGGTGGTGGTGGTGG TTGTGGTGGTGG
P7	HS/TTTUCUGAUUCAACUUUGGUGATT/Azo/GAGACTTTGGTGGTGGTGGTGGTT GTGGTGGTGG

4. Molecular structure characterization data

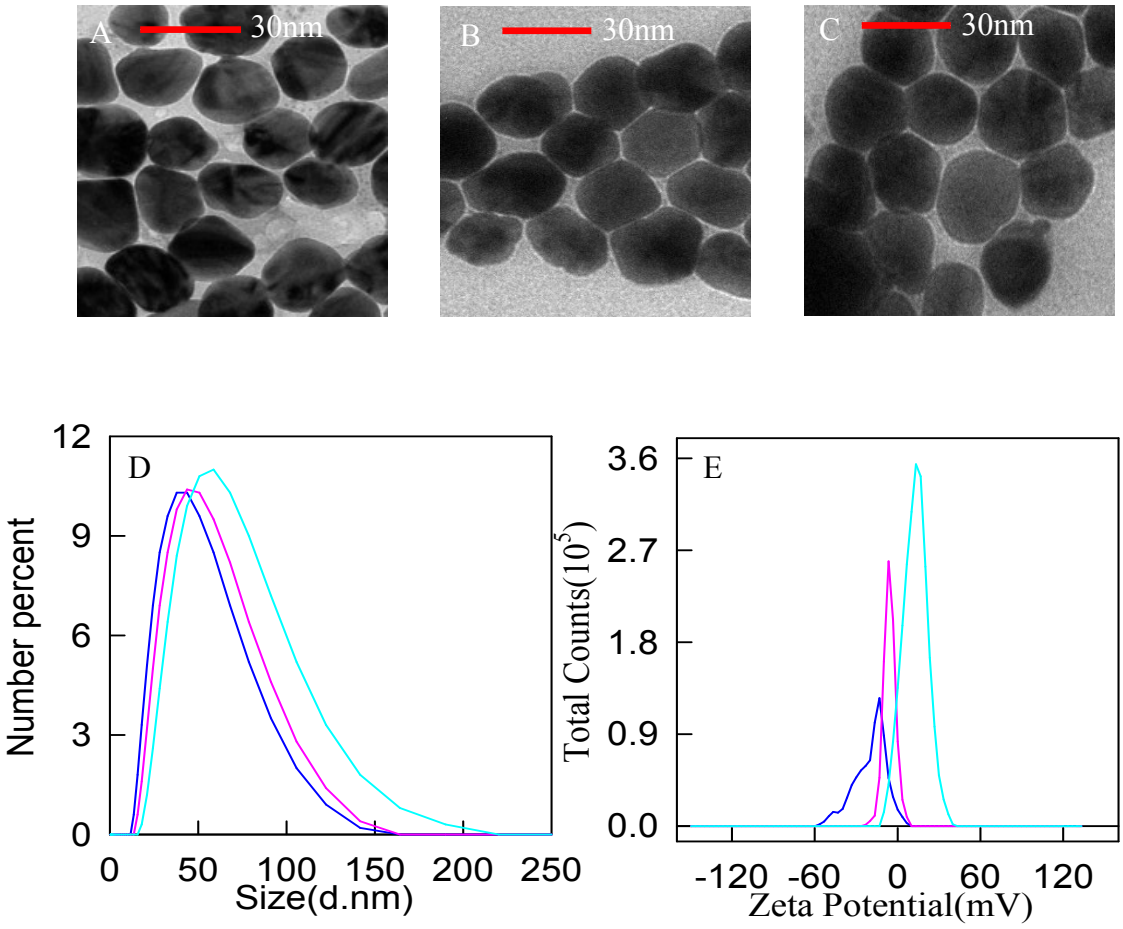


Fig. S1 TEM images of (A) AuNPs and (B) DDS, (C) DDS@TMPyP4 (D) Size and (E) Zeta Potential of AuNPs (blue curve), βCD-AuNPs (pink curve) and DDS (Lake blue curve).

5. Feasibility study

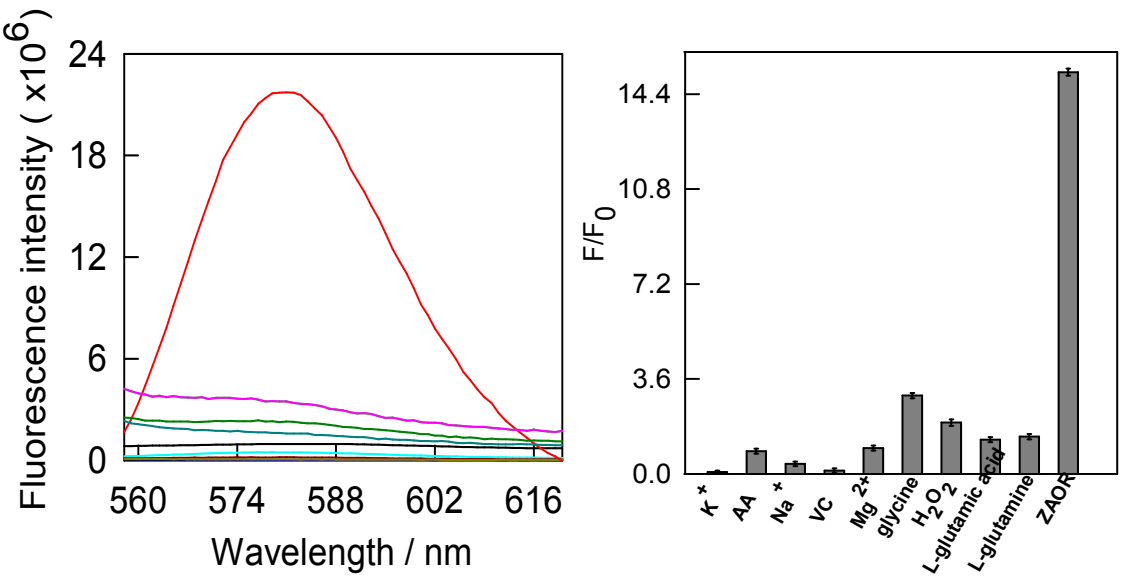


Fig. S2 Fluorescence responses of DDS in the presence of NADPH (50 μM) to various species: KCl (10 mM), AA (1 mM), NaCl (10 mM), vitamin C (1 mM), $MgCl_2$ (10 mM), glycine (10 mM), H_2O_2 (1 mM), L-glutamic acid (10 mM), L-glutamine (10 mM), rat liver microsomes (21 μg·mL^{-1}).

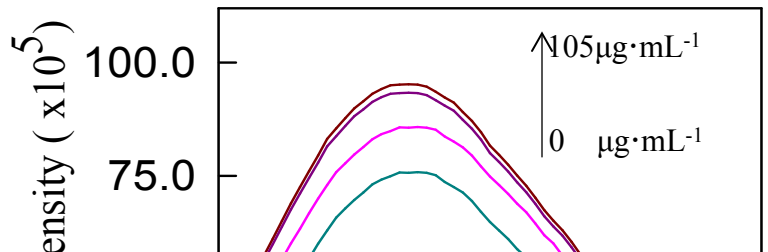
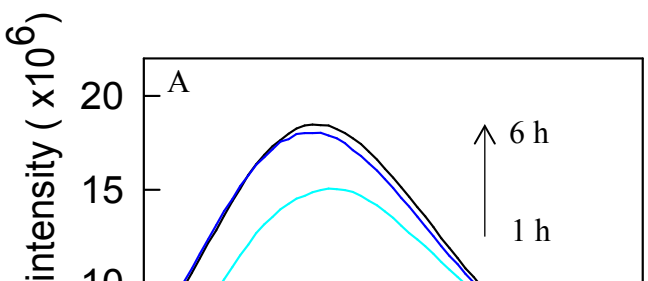


Fig. S3 Fluorescence emission spectra of DDS reacted with different concentrations of rat liver microsomes and NADPH (50 μM) at 37 ℃ for 6 h in buffer solutions (The arrow indicated the concentration: 0, 10.5, 21, 42, 52, 63, 73.5, 84, 94.5, 105 μg·mL^{-1}, pH 7.4) under hypoxic condition.



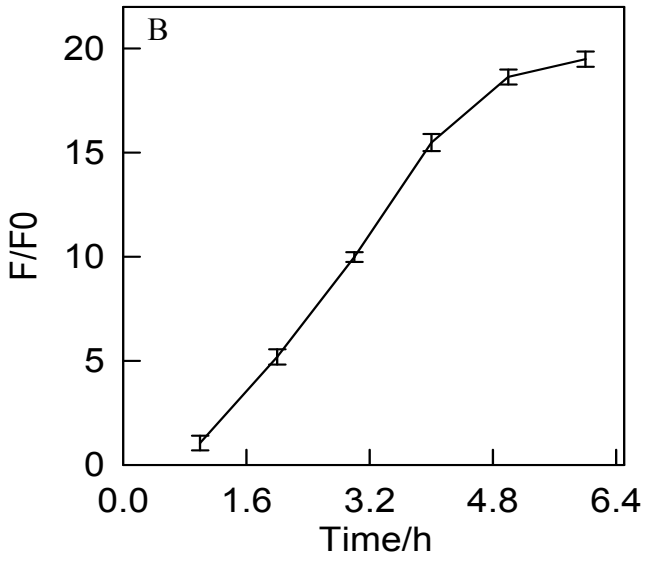
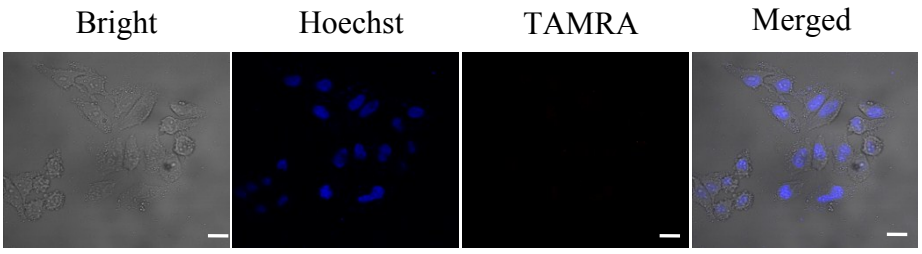


Fig. S4 (A) Fluorescence emission spectra of DDS with varied reaction time (1, 2, 3, 4, 5, 6 h) in the presence of rat liver microsomes (21 μg·mL^{-1}) and NADPH (50 μM) under hypoxic condition. (B) Calibration curve of fluorescence signal enhancement (F/F0, where F and F0 represents the fluorescence intensity of TAMRA after and before rat liver microsomes addition with varied reaction time (1, 2, 3, 4, 5, 6 h) under hypoxic conditions) versus concentration.



(a)

(b)

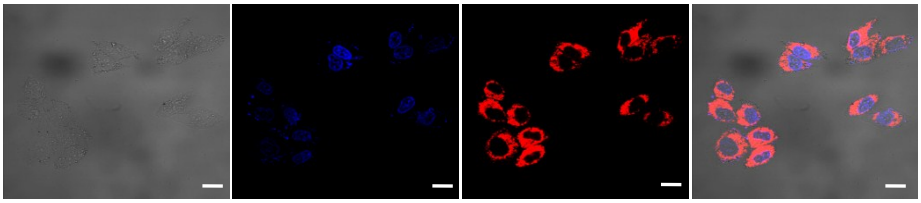


Fig. S5A Confocal microscopy images of HepG2 cells incubated with (a) FITC-labeled random DNA and (b) FITC labelled-AS1411 aptamer-fabricated DDS at 37 ℃ for 1 h, following by incubated at 37 °C atmosphere for 3 h. (Blue color: nuclei stained by Hoechst 33342, red color: TAMRA). Scale bar: 10 μm.

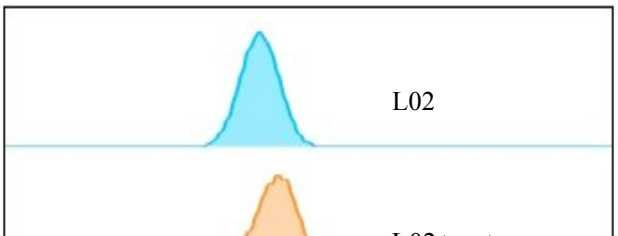


Fig. S5B Flow cytometry analysis of the HepG2 cells and L02 cells incubated with FITC labelled-AS1411 aptamer-fabricated DDS (250 nM) for 2 h. The concentration was defined by FITC labelled-AS1411.

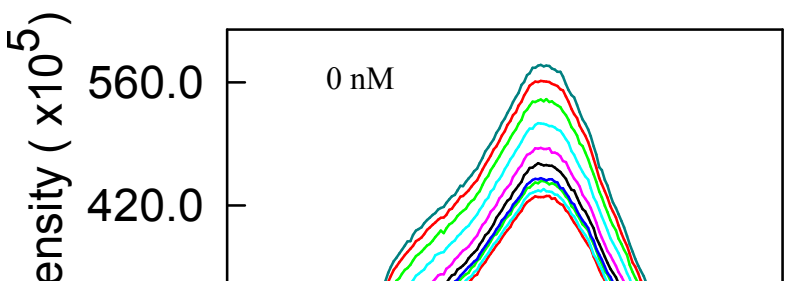


Fig. S6 Fluorescence emission spectra of 10 μM TMPyP4 in PBS buffer upon addition of increasing concentrations of DDS. The arrow indicated the concentration of DDS was 0-1.1 nM. The concentration of DDS was defined by AuNPs.

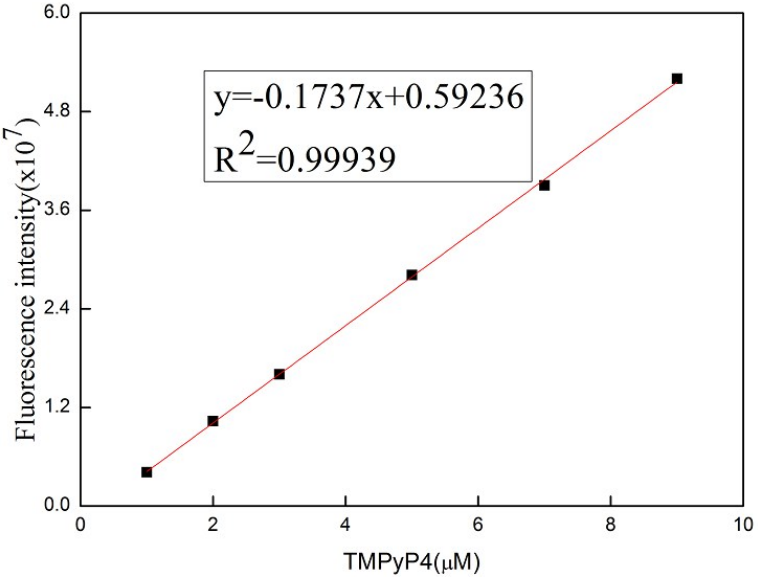


Fig. S7 The standard curve of TMPyP4.

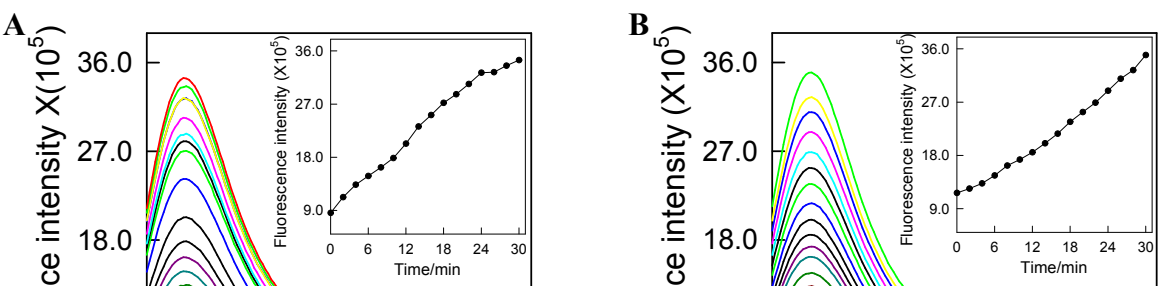


Fig. S8 The determination of ROS from (A) TMPyP4 (B) DDS@TMPyP4 upon different illumination time.

5. Cell imaging

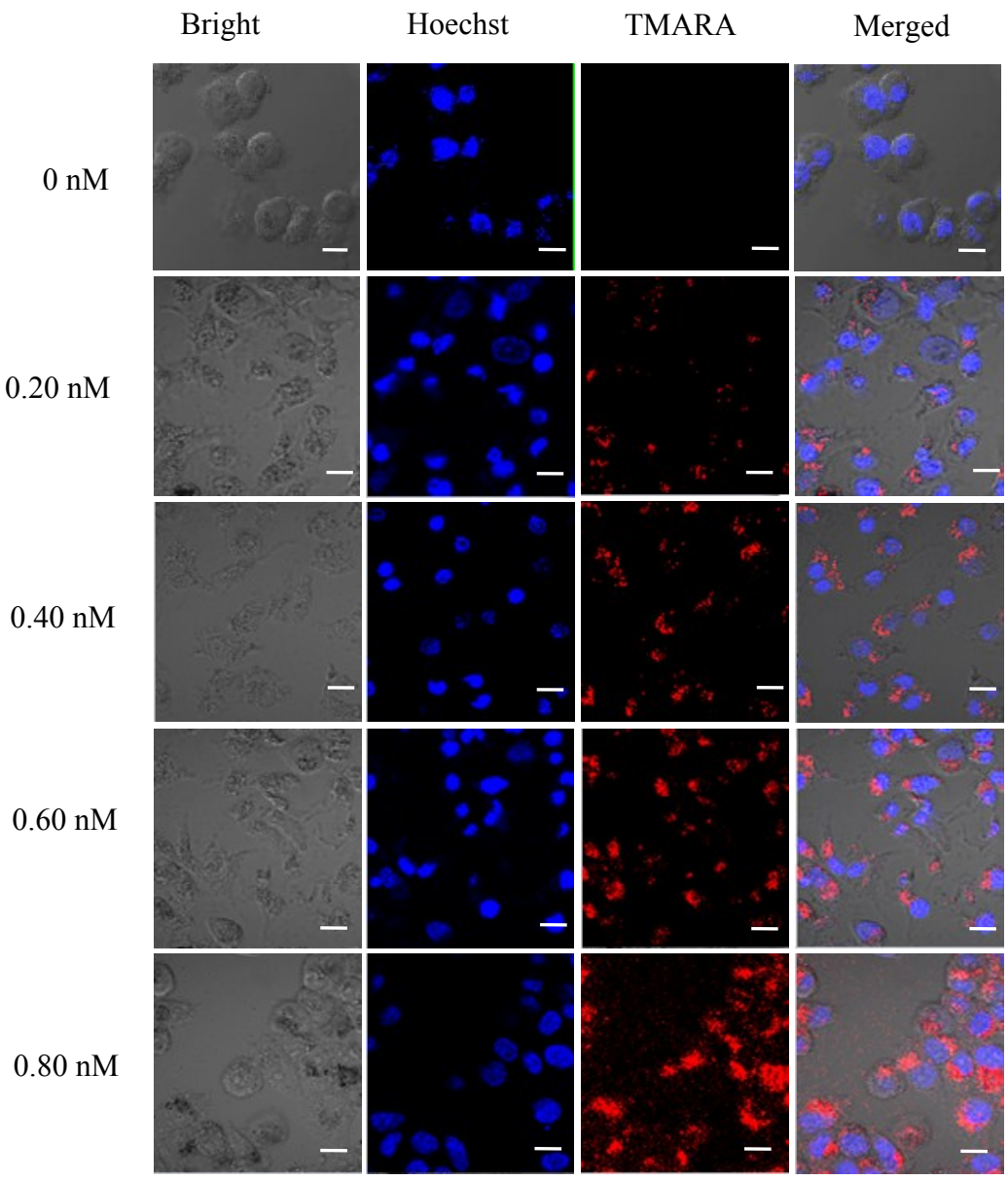


Fig. S9 Confocal fluorescence images of HepG2 cells under mild hypoxia (oxygen concentration ~10%) incubated with DDS@TMPyP4 for different concentrations (0, 0.20, 0.40, 0.60, 0.80 nM) at 37 ℃ for 1 h, following by irradiated (660 nm, 1 W·cm^{-2}) for 30 min, and then incubated at 37 °C atmosphere for 3 h. (Blue color: nuclei stained by Hoechst 33342, red color: TAMRA). Scale bar: 10 μm. The concentration was defined by AuNPs.

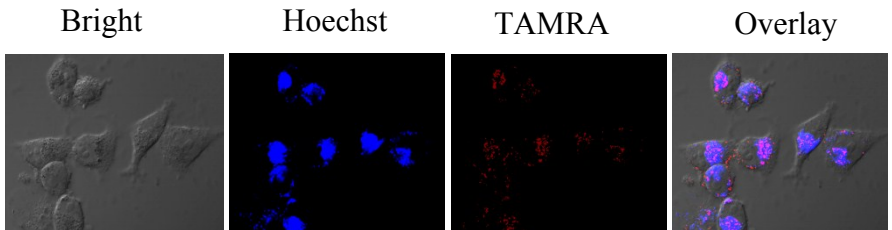


Fig. S10 Confocal fluorescence images of HepG2 cells under mild hypoxia (oxygen concentration ~10%) incubated with DDS@TMPyP4 for 1 h, following by irradiated (660 nm, 1 W·cm^{-2}) for 30 min, and then incubated at 37 °C for different reaction time (0, 1, 2, 3, 4 h). (Blue color: nuclei stained by Hoechst 33342, red color: TAMRA). Scale bar: 10 μm.

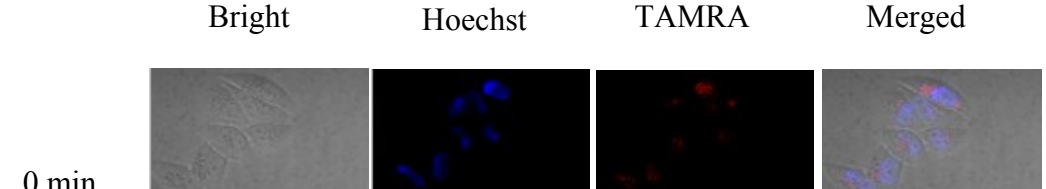
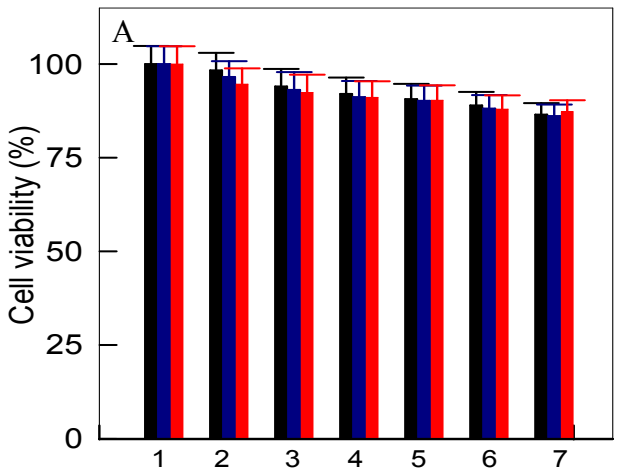


Fig. S11 Confocal fluorescence images of HepG2 cells under mild hypoxia (oxygen concentration ~10%) incubated with DDS@TMPyP4 for 1 h, following by irradiated (660nm, 1 W·cm^{-2}) with different times (0, 10, 20, 30 min), and then incubated at 37 °C atmosphere for 3 h. (Blue color: nuclei stained by Hoechst 33342, red color: TAMRA). Scale bar: 10 μm.

6. Cytotoxicity study



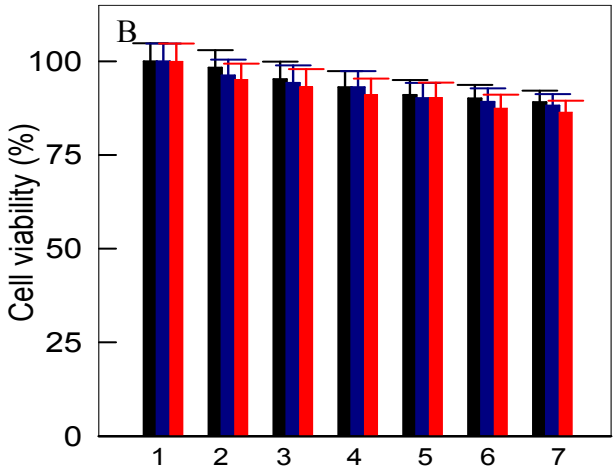


Fig. S12 Cell Viability of HepG2 cells(A) and L02 cells (B) which were incubated with DDS-A(black bars), DDS-B (blue bars) and DDS(red bars) at various concentrations (nM) for 1 h, following by irradiated (660 nm, 1 $W \cdot cm^{-2}$) with 30 minutes, and then incubated at 37 °C atmosphere for another 48 h. 1-7 represents the concentration of DDS-A, DDS-B, DDS: 0, 0.14, 0.28, 0.42, 0.56, 0.70, 0.84 nM, respectively. The concentration was defined by AuNPs.

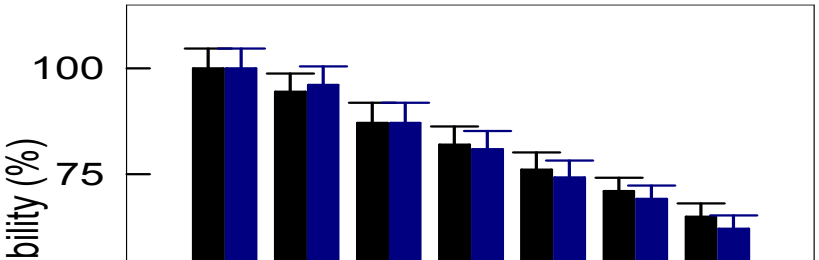
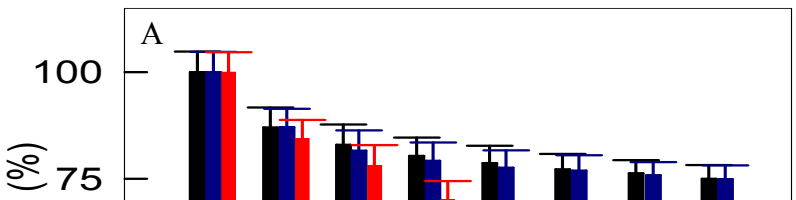


Fig. S13 Cell Viability of HepG2 cells (black bars) and L02 cells (blue bars) incubated with free TMPyP4 at various concentrations (nM) for 1 h, following by irradiated (660 nm, 1 W·cm^{-2}) with 30 minutes, and then incubated at 37 °C atmosphere for another 48 h. 1-7 represents the concentration of free TMPyP4: 0, 0.12, 0.24, 0.36, 0.48, 0.60, 0.72 nM, respectively.



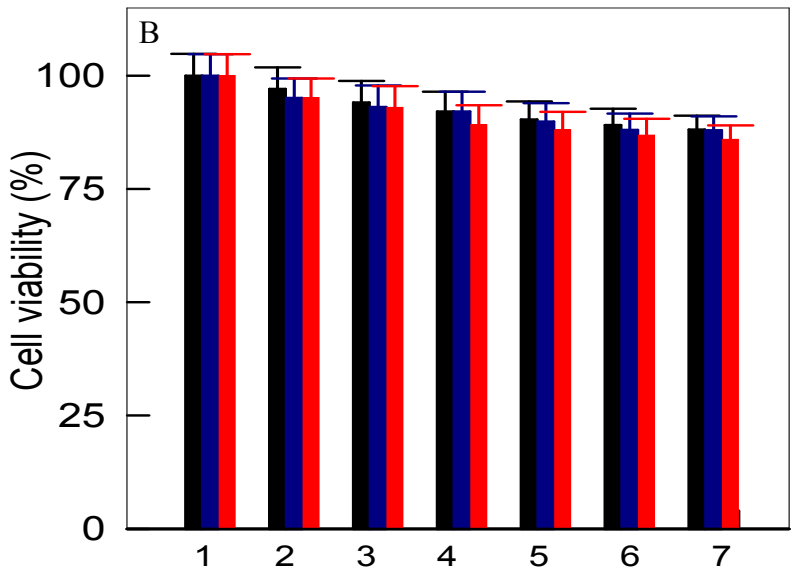


Fig. S14 Cell Viability of HepG2 cells(A) and L02 cells(B) under mild hypoxic condition (10%) incubated with DDS-A(black bars), DDS-B (blue bars) and DDS(red bars) at various concentrations (nM) for 1 h, and then incubated at 37 °C atmosphere for another 48 h. 1-7 represents the concentration of DDS-A, DDS-B and DDS: 0, 0.14, 0.28, 0.42, 0.56, 0.70, 0.84 nM, respectively. The concentration was defined by AuNPs.

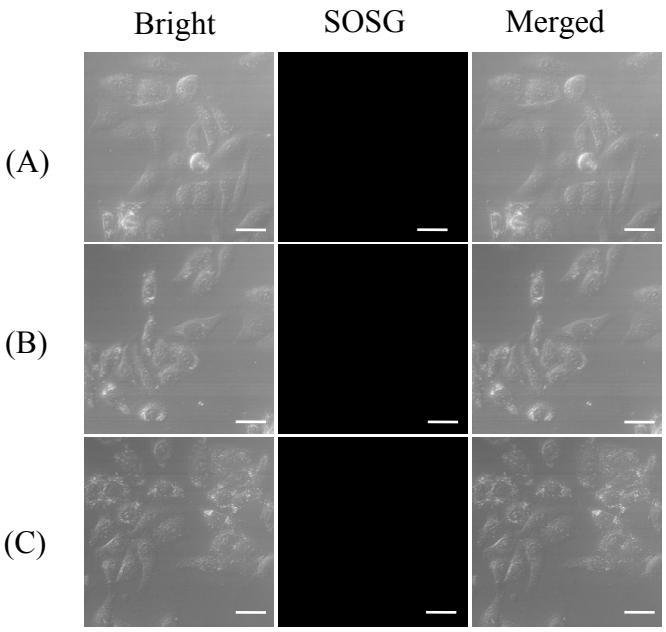


Fig. S15 HepG2 cells incubated with DDS (A), DDS-A(B) and DDS-B(C) for 1 h and fixed with 4% paraformaldehyde for 20 min followed by adding 10 μL of SOSG working solution and incubated for 30 min. Then, the cells were irradiated for 30 min. The concentration was defined by AuNPs (0.80 nM). Scale bar: 20 μm.

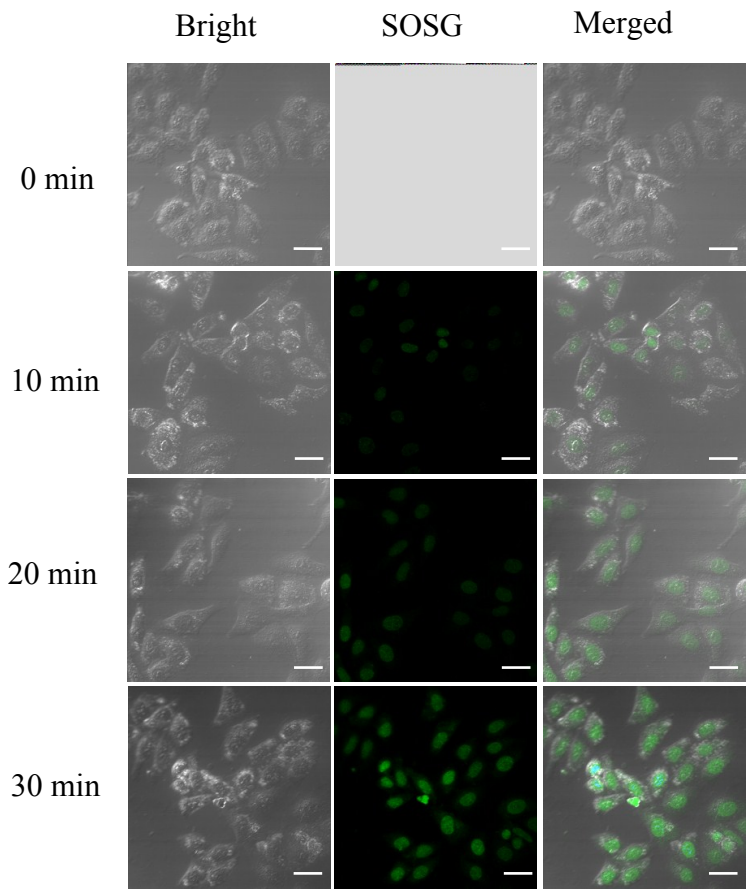


Fig. S16 HepG2 cells incubated with DDS@TMPyP4 for 1 h and fixed with 4% paraformaldehyde for 20 min followed by adding 10 μL of SOSG working solution and incubating for 30 min. Then, the cells were irradiated for different time. The concentration was defined by AuNPs (0.80 nM). Scale bar: 20 μm.