Supporting Information

Core-shell metal-organic frameworks with pH/GSH dual-responsiveness for combined chemo-chemodynamic therapy

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1. Materials and methods

Materials

All reagents and chemicals were commercially available and of analytical grade for use directly without further purification. Copper nitrate trihydrate (99%) were purchased from Aladdin-Reagent Co. Ltd. (China). 3-amino-1,2,4-triazole (3-AT, 98%), Hyaluronic acid (HA) and doxorubicin (DOX) were purchased from HEOWNS-Reagent Co. Ltd. (Tianjin). N-Hexadecyltrimethylammonium chloride (CTAC) and Bis[3-(triethoxysilyl)propyl]tetrasulfide (BTES) (90%) were purchased from Rhawn Chemical Reagent Co. (Shanghai), Fetal bovine serum (FBS) was purchased from Thermo-Fisher Scientific (Waltham, USA). The reduced GSH assay kit was purchased from Beijing Solarbio Science & Technology Co. Ltd. CAT and MDA assay kit were purchased from Suzhou Michy Biomedical Technology Co. Ltd. Liperfluo fluorescence probe was purchased from DOJINDO Co. Ltd. Human liver hepatocellular carcinoma HepG2, and human liver HL7702 were obtained from KeyGEN BioTECH Co. (Nanjing, China).

2. Synthesis of Cu-MOF and Cu-MOF@SMON/DOX-HA

Cu-MOF@SMON nanoparticles (NPs) were prepared according to the previous procedure.[1-3] Firstly, $Cu(NO_3)_2 \cdot 3H_2O$ (0.25 mmol, 60 mg) and 3-AT (0.25 mmol, 21 mg) were mixed in 4 mL of mixed solvent (V_{DMF}:V_{H2O} =1:2) with sonication, then transferred to a Teflon-lined autoclave (15 mL) and heated to 130 °C for 24 h, where the metal ions were coordinated with ligands using the O-N bonds. Subsequently slowly cooled ambient temperature, the rod-like Cu-MOF nanoparticles (NPs) were obtained by centrifugal separation and washed with DMF and H_2O. Cu-MOF@SMON was prepared according to the previous procedure. Typically, the Cu-MOFs were immersed in 80% methanol aqueous solution for 3 h, then centrifuged and 20 mg of them were dispersed in 10 mL ethanol containing CTAC (30 mg), the 400 μL of concentrated ammonia was added with sonication. Finally, 300 μL methanol solution containing BTES (60 μL) was added to the above solution for three times (30 min interval) and stirred at room temperature for 12 h. The Cu-MOF@SMON was separated by centrifugation and washed with ethanol and acetone three times. As for Cu-MOF@SMON/DOX-HA, stock solutions of HA (10 mg mL^{-1}) and DOX (5 mg mL^{-1}) were prepared in ultrapure water. The 10 mg of Cu-MOF@SMON was dispersed in 7 mL ultrapure water, then 1

mL HA stock solution was added with sonication for 20 min, after which the mixed solution was stirred at room temperature for 16 h. Subsequent centrifugation obtained Cu-MOF@SMON-HA and was re-dispersed in 5 mL of ultrapure water, the 2 mL DOX stock solution was added to above solution with sonication for 30 min, finally the mixed solution was stirred at room temperature for 24 h, and Cu-MOF@SMON/DOX-HA was obtained by centrifugation and washed with ultrapure water. The DOX loading efficiency (DLE %) on Cu-MOF@SMON/DOX-HA was defined as follows:

$$DLE\% = \frac{m_{(the\ total\ of\ DOX)} - m_{(the\ supernant\ of\ DOX)}}{m_{(the\ total\ of\ Cu-MOF@SMON/DOX-HA)}} \times 100\%$$

3. Characterization

The UV-vis absorption spectra were collected by using a UV-vis spectrophotometer (Shimadzu 1750, Japan). SEM images were obtained from FEI Nova Nano SEM-450. TEM images were obtained from FEI TECNAI G2 SPIRIT BIO. The dynamic light scattering (DLS) and zeta potential experiments were characterized by Zetasizer Nano-ZS (Malvern Instruments). The confocal laser microscope (CLSM) data were obtained using a CLSM (Andor REVOLUTION WD). Flow cytometry data were obtained from BD FACSAria™ III.

4. DOX released from Cu-MOF@SMON/DOX-HA NPs

2 mg of Cu-MOF@SMON/DOX-HA NPs were dispersed in 3 mL of different PBS buffer solution (pH: 7.4, pH: 5.0, pH: 7.4 + GSH: 10 mM) and stirred for different times (0.5, 1, 2, 4, 6, 8, 12 and 24 h). Next, the supernates were collected by centrifugation and correspongding absorbances at 480 nm were determined by spectrophotometer to evaluate the release efficiency of DOX.

5. Detection of extracellular ·OH production and GSH depletion

The generation of ·OH was detected by methylene blue (MB) decoloration experiment and electron spin resonance (ESR) assay, where ESR assay used 5,5-dimethyl-1-pyrroline N-oxide (DMPO) as a spin trapping agent. Typically, 1 mL of NPs (100 μg mg^{-1}), 100 μL of MB (20 μg mg^{-1}), 500 μL of H_2O_2 (20 mM) and 400 μL of GSH (20 mM) solution dealt with different conditions (pH = 7.4: (a) MB; (b) MB + H_2O_2; (c) MB + H_2O_2 + NPs; (d) MB + H_2O_2 + NPs + GSH, pH = 5.0: (a) MB; (b) MB + H_2O_2; (c) MB + H_2O_2 + NPs; (d) MB + H_2O_2 + NPs + GSH) were well-mixed.

Then, Time–dependent fluorescence intensity at 680 nm was monitored and evaluated generation of ·OH. For electron spin resonance (ESR) measurements, 10 μL of 5,5-dimethyl-1-pyrroline N-oxide (DMPO, 1 M) was mixed with 1 mL solution containing Cu-MOF@SMON/DOX-HA (100 μg mL^{-1}), GSH (10 mM), H_2O_2 (10 mM) and then the mixture was transferred into a quartz capillary and measured on an EMXplus spectrometer (Bruker, Germany).

GSH depletion was examined by reduced GSH assay kit. The 1 mL of GSH (20 mM) and 1 mL of NPs solutions with different concentrations (25, 50, 100, 200, 300 and 400 μg mg^{-1}) were mixing and carried out under different conditions for 3 h and 6 h, respectively. Then, the mixed solution was centrifuged and 20 μL of supernatant was collected, to which 140 μL of reagent II and 40 μL of reagent III were added. After 2 min of reaction, the fluorescence intensity at 412 nm was collected to evaluate the consumption of GSH.

6. Cell culture and in vitro cytotoxicity evaluation

The Human Liver Cell Lines HL7702 (HL7702) and human hepatocellular carcinoma HepG2 (HepG2) cells were cultured in 1640 supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37 °C with 5% CO_2. After 10 days of culture, the HL7702 and HepG2 cells were collected and then cultured for further experimental use.

The in vitro cytotoxicity of Cu-MOF@SMON/DOX-HA was evaluated by the MTT assays with HL7702 and HepG2 cells. First, HL7702 and HepG2 cells were incubated for 24 h. Then the cells were treated with different samples for 12 h: a) control; b) free DOX (0, 0.681, 1.362, 2.725, 5.45 and 10.9 μg mL^{-1}); c) Cu-MOF@SMON-HA NPs (0, 3.125, 6.25, 12.5, 25 and 50 μg mL^{-1}); d) Cu-MOF@SMON/DOX-HA NPs (0, 3.125, 6.25, 12.5, 25 and 50 μg mL^{-1}). After removing the medium, the cells were washed thrice with PBS and further cultured for another 24 h or 48 h by 1640 medium. Finally, 20 μL of MTT solution was added and cultured for another 4h. Each well was added with the dimethyl sulfoxide (DMSO, 100 μL) and tested by a microplate reader at the wavelength of 490 nm.

7. Cellular uptake behaviors of Cu-MOF@SMON/DOX-HA NPs

HepG2 cells were seeded into a 6-well plates for 24 h, then Cu-MOF@SMON/DOX-HA (25 μg·mL^{-1}) was adding and cultured with different times. After incubation, the cells were washed with

PBS for three times and incubated with Hoechst 33258 for 20 min to stain nucleus for confocal fluorescence images obtained by CLSM. In addition, the uptake of Cu-MOF@SMON/DOX-HA NPs was also measured by flow cytometry.

8. Detection of intracellular ROS production and GSH consumption

HepG2 cells were seeded into a 6-well plates for 24 h, then the HepG2 cells were sequentially treated with different samples for 6 h: a) control; b) free DOX (5.45 μg mL^{-1}); c) Cu-MOF@SMON-HA (25 μg mL^{-1}); d) Cu-MOF@SMON/DOX-HA (25 μg mL^{-1}). After removing the residual nanomaterials, 1 mL of DCFH-DA (10 μM) was added to each wells and cultured for another 15 min. Finally, the cells were washed thrice with PBS and fluorescence images were recorded by using CLSM.

The intracellular GSH consumption was detected using reduced GSH assay kit. The HepG2 cells were seeded into a 25 cm^2 culture flasks for 24 h, then the cells were sequentially treated with different samples for 9 h: a) control; b) free DOX (5.45 μg mL^{-1}); c) Cu-MOF@SMON-HA (25 μg mL^{-1}); d) Cu-MOF@SMON/DOX-HA (25 μg mL^{-1}). After removing the medium, the cells were collected and frozen-thawed thrice using liquid nitrogen and 37 °C of water. Finally, the samples were centrifuged and the supernatant was used for GSH assay.

9. Live/dead cells staining and apoptosis detection assay

HepG2 cells were seeded into a 6-well plate with a density of 1.0×10^5 cells per well for 24 h, then the HepG2 cells were sequentially treated with different samples for 24 h: a) control; b) free DOX (5.45 μg mL^{-1}); c) Cu-MOF@SMON/DOX-HA (25 μg mL^{-1}). After removing the medium, the cells were stained by calcein-AM (2 μM) and PI (4 μM) as well as then analyzed using a CLSM.

For cell apoptosis, HepG2 cells were cultured with different formulations (PBS, DOX: 5.45 μg mL^{-1}, Cu-MOF@SMON/DOX-HA: 25 μg mL^{-1}) for 24 h. Afterwards, all of the treated cells were collected, washed, stained with FITC/PI for 20 min, and fluorescence analysis by flow cytometry.

10. CAT activity assay

The CAT activity was detected by the CAT assay kit, and the activity of CAT in groups CAT+H_2O_2, CAT+H_2O_2+3-AT and CAT+H_2O_2+NPs (H_2O_2: 10 Mm, CAT: 1 mg mL^{-1}, NPs: 50 μg

mL^{-1}) were assayed at 37 °C and pH = 7.0, respectively. The final assay and calculations were performed according to the CAT monitoring instructions. Catalyzing the degradation of 1 μmol H2O2 per minute per ml of serum is defined as one unit of enzyme activity (As follows).

$$CAT\ (\mu mol/min/mL) = (\Delta A\text{-}0.0042) \div 0.0776 \times V_1 \div V_2 \div T = 11.47 \times (\Delta A\text{-}0.0042)$$

ΔA: $A_{control} - A_{experiment}$, V_1: Total volume of reaction system, V_2: Added sample volume, T: reaction time of 10 min.

11. Detecting intracellular MDA

For the MDA assay, HepG2 cells were firstly seeded into 6-well plates with fresh DMEM containing 10% FBS (2 mL per well). After 24 h, the media were removed and replaced with different (DMEM, DOX: 10.88 μg mL^{-1}; Cu-MOF@SMON-HA:50 10.88 μg mL^{-1}; Cu-MOF@SMON/DOX-HA:10 μg mL^{-1}) of NPs and incubated for another 24 h. Then the culture solution was removed and the cells were collected for MDA analysis.

11. Fig. S1-S8

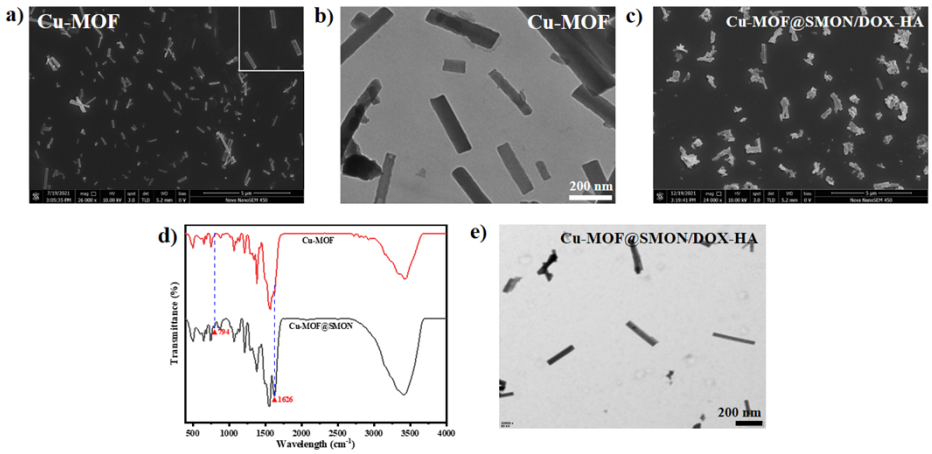

Figure S1: (a) SEM images of Cu-MOF (Scale bar: 5 μm), (b) TEM images of Cu-MOF (Scale bar: 200 nm), (c) SEM images of Cu-MOF@SMON/DOX-HA (Scale bar: 500 nm), (d) FTIR spectra of Cu-MOF and Cu-MOF@SMON, (e) TEM images of Cu-MOF@SMON/DOX-HA (Scale bar: 200 nm).

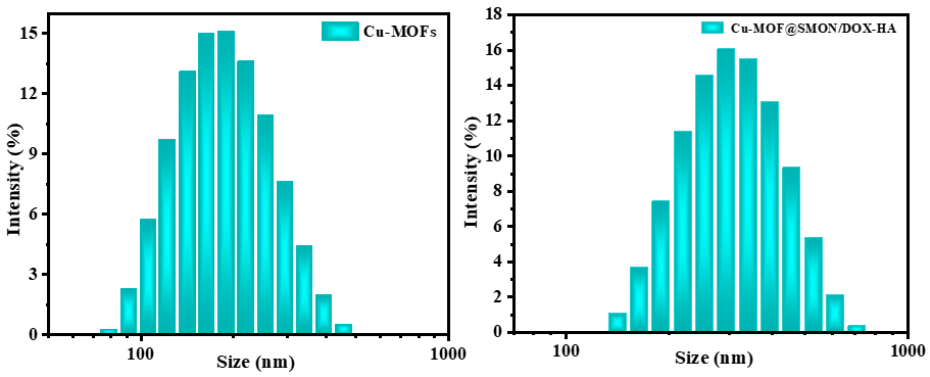

Figure S2: DLS data of Cu-MOFs (205±15 nm, PDI: 0.217) and Cu-MOF@SMON/DOX-HA (300±15 nm, PDI: 0.265).

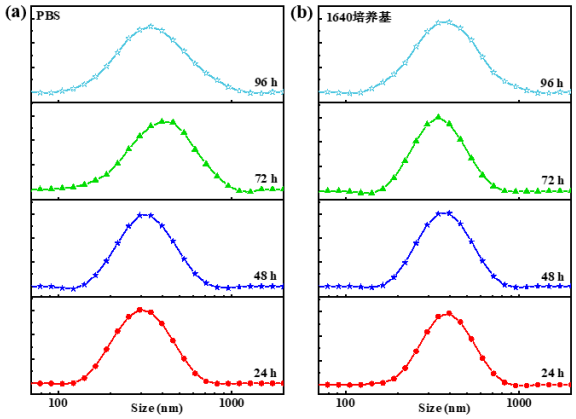

Figure S3: (a) the hydrodynamic size of Cu-MOF@SMON/DOX-HA in PBS for 4 days, (b) the hydrodynamic size of Cu-MOF@SMON/DOX-HA in 1640 medium with FBS for 4 days.

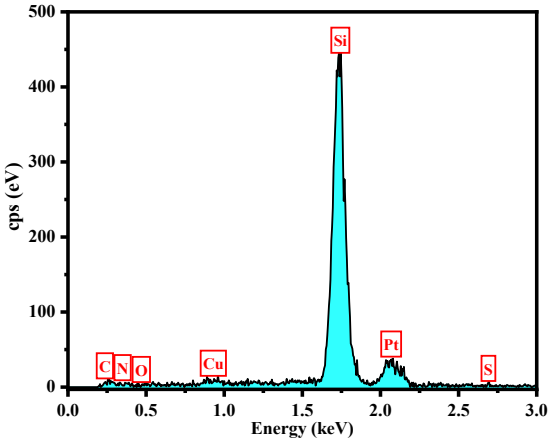

Figure S4: energy-dispersive spectroscopy survey scan of Cu-MOF@SMON.

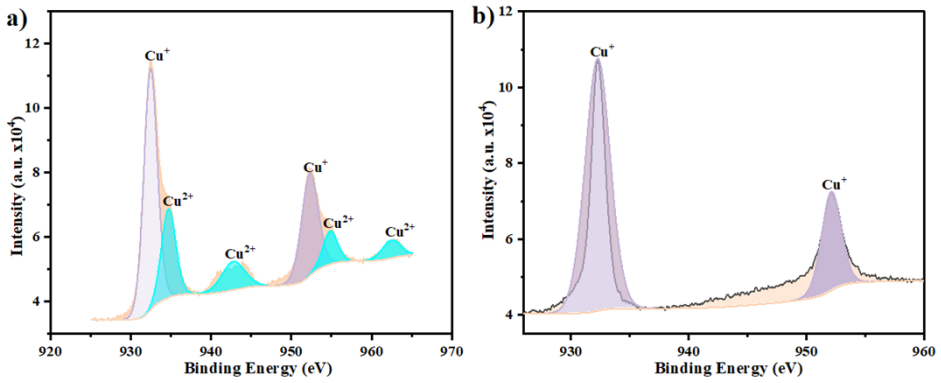

Figure S5: a) XPS survey scan of Cu-MOF@SMON without GSH. b) XPS survey scan of Cu-MOF@SMON with GSH.

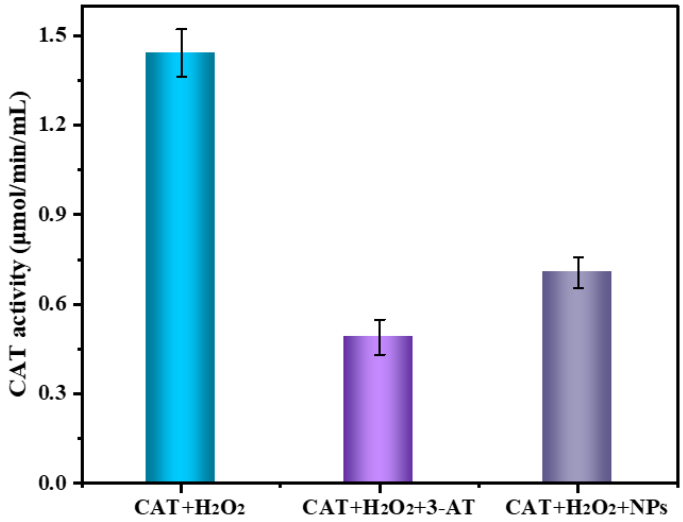

Figure S6: CAT activity was measured by the CAT kit (n=3).

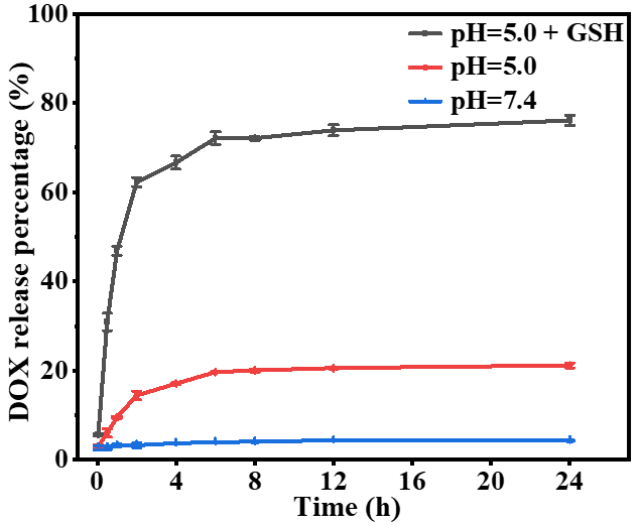

Figure S7: DOX release from Cu-MOF@SMON/DOX-HA in PBS at different pH values (n=3).

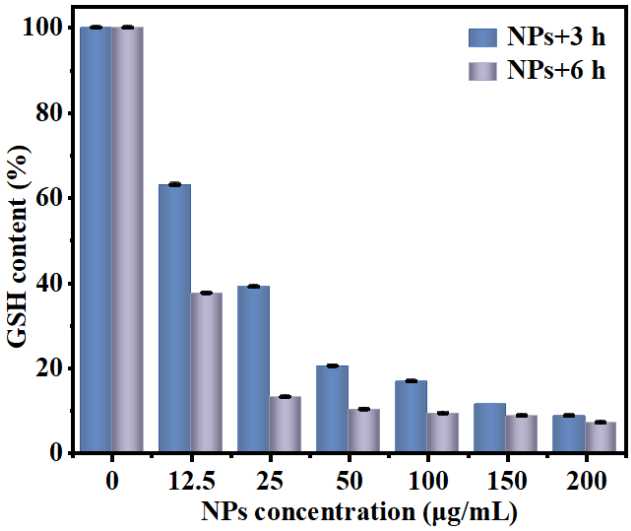

Figure S8: Consumption of GSH in extracellular under incubation of different times (n=3)

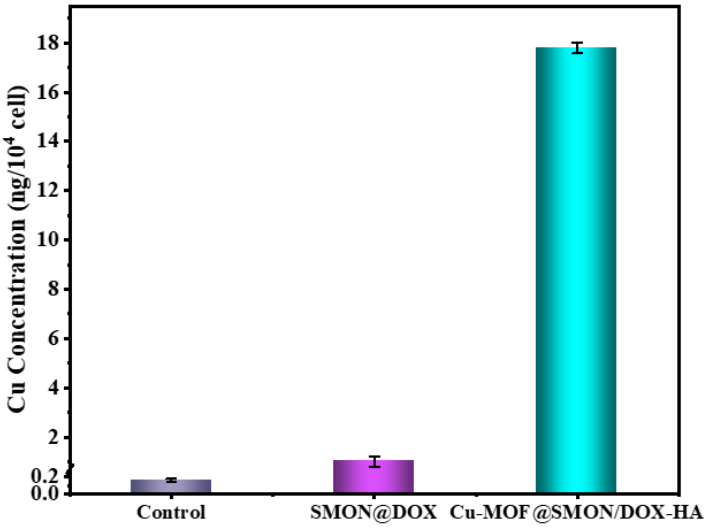

Figure S9: Cellular uptake of Cu-MOF@SMON/DOX-HA in HepG2 cells by ICP-MS.

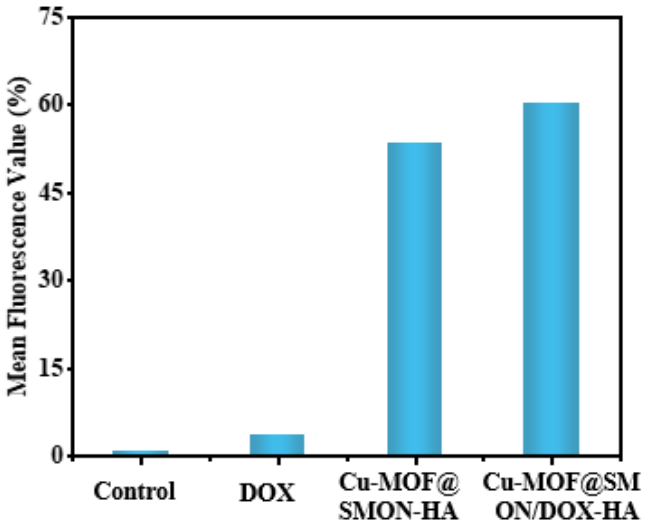

Figure S10: Mean fluorescence intensity of ·OH-generating in HepG2 cells.

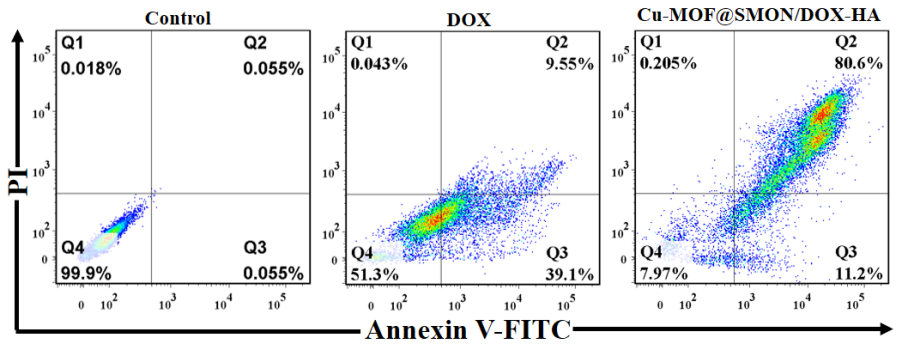

Figure S11: Flow cytometry analysis of HepG2 cells incubated with different formulations.

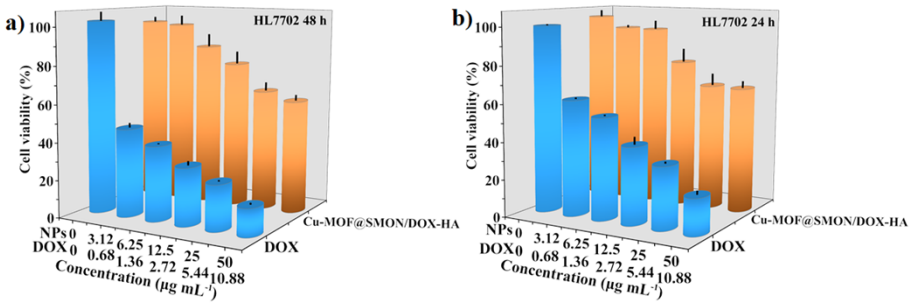

Figure S12: Cell viability of HL7702 cells with different treatments under various concentrations of nanoparticles for 24 h a); or 48 h b)

12. References

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