

## Supporting Information

### **Ascorbic acid induced HepG2 cells' apoptosis *via* intracellular reductive stress**

Xiaonan Gao,<sup>#</sup> Keyan Wei,<sup>#</sup> Bo Hu, Kehua Xu\* and Bo Tang\*

College of Chemistry, Chemical Engineering and Materials Science, Collaborative Innovation Center of Functionalized Probes for Chemical Imaging in Universities of Shandong, Key Laboratory of Molecular and Nano Probes, Ministry of Education, Shandong Provincial Key Laboratory of Clean Production of Fine Chemicals, Shandong Normal University, Jinan 250014, P. R. China

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## Experimental Procedures

### 1. Materials and Characterizations

#### 1.1 Materials

3-Mercaptopropionic acid (MPA), cadmium chloride ( $\text{CdCl}_2$ ), sodium borohydride ( $\text{NaBH}_4$ ), tellurium (Te), tetraethyl orthosilicate (TEOS), ascorbic Acid (AA), dehydroascorbic acid (DHA), tetrahydrofuran (THF) were purchased from China National Pharmaceutical (Shanghai, China). (3-aminopropyl) trimethoxysilane (APS), hexadecyl trimethyl ammonium bromide (CTAB), N-hydroxysulfosuccinimide sodium salt (sulfo-NHS) and 1-ethyl-3-(3-(dimethylamino)-propyl) carbodiimide hydrochloride (EDC) were purchased from Alfa Aesar (Tianjin, China). Poly(2-vinylpyridine)-polyethylene glycol-folic acid (PPF) was synthesized from Xin Qiao Biotechnology (Hangzhou, China). 3-(4, 5-Dimethyl-2-thiazolyl)-2, 5-diphenyl-2-H-tetrazolium bromide (MTT) was purchased from Sigma. The NAD(P)H probe (DCI-MQ) is synthesized by our group following previous report. All chemicals and solvents were used as received without purification unless stated. Ultrapure water of  $18.2 \text{ M}\Omega\cdot\text{cm}$  was obtained from a millipore water ultrapurification system. Annexin V-FITC/PI Apoptosis Detection Kit was purchased from Jiamei Biological Company (Beijing, China).

#### 1.2 Characterizations

Transmission electron microscopy (TEM) was performed on a JEM-100CX-II electron microscope. UV-Vis absorption analysis was carried out on a UV-1700-vis spectrometer (Shimadzu, Japan). All pH values were measured by a pH-3c digital pH meter (LeiCi, China) with a combined glass-calomel electrode. Fluorescence spectra were recorded on an Edinburgh FLS-980 fluorescence spectrophotometer. Absorbance was measured in a microplate reader (Synergy 2, Biotek, USA) in the MTT assay. Confocal fluorescence imaging was performed with a TCS SP8 confocal laser scanning microscopy (CLSM, Leica Germany) with an objective lens ( $\times 63$ ). Cells were analysed in flow cytometer. Anaero Pack-Anaero and Aero (Mitsubishi Gas Chemical company, Inc.) were used to provide a hypoxic cell culture environment. *In vivo* fluorescence imaging was conducted on the *in vivo* imaging system (IVIS, PerkinElmer).

### 2. Preparation of Nanocarriers

#### 2.1 Preparation of CdTe QDs Capped with MPA.

The preparation of CdTe quantum dots followed hydrothermal synthesis. Typically, 70.95 mg  $\text{NaBH}_4$  was dissolved in 3 mL of  $\text{H}_2\text{O}$ ,  $\text{N}_2$  gas was then bubbled into the solution for 30 min at  $4^\circ\text{C}$ . Afterwards, 95.7 mg of Te was added fastly into the solution under vagnous stirring, followed by 12 h reaction under  $\text{N}_2$  atomsphere at  $4^\circ\text{C}$ . The solution turned to light purple color, indicating the formation of NaHTe solution.



Consequently, 30 mL of  $\text{H}_2\text{O}$  was bubbled  $\text{N}_2$  for 30 min, followed by addition of  $\text{CdCl}_2$  (109.99 mg) and MPA (48 mM, 126.75  $\mu\text{L}$ ) with  $\text{N}_2$  protection at  $4^\circ\text{C}$ . Then NaOH (1 M) was applied to adjust pH to 9.0 before quickly injecting 0.5 mL freshly prepared NaHTe solution, the mixture solution was refluxed at  $100^\circ\text{C}$  for 1 h and measured through fluorescence spectrometer to obtain the excitation/emission wavelength. The as-prepared CdTe solution was concentrated to 5 mL, and the CdTe QDs were precipitated with 2-propanol followed by centrifugation.

### 3. Drug loading and release

5 mg CTMP was dissolved in 5 mL N<sub>2</sub>-saturated citrate buffer (pH = 2.0) and then 5 mg of AA was added. With continuous N<sub>2</sub> flow, the mixture was stirred in dark at 4 °C for 24 h to ensure the maximum loading capacity. The prepared CTMP-AA was centrifuged at 14,000 rpm for 15 min at 4 °C. After the supernatant removal, the precipitate was washed in SSC buffer (pH = 7.4) for several times to get rid of any physisorbed AA. The absorption intensity of the supernatant ( $\lambda_{\text{max}} = 246 \text{ nm}$ ) was measured by UV-Vis spectrometer.

Two SSC buffer solutions with different pH values of 6.0 and 7.4 were firstly prepared. CTMP-AA were dispersed in 5 mL of each buffer and subsequently placed at 37 °C with shaken at 120 rpm with continuous N<sub>2</sub> flow. During different time intervals, 0.5 mL of solution was withdrawn every time from the solution to obtain the UV-Vis absorption. The volume of the release medium kept constant by adding 0.5 mL fresh medium after each sampling.

### 4. General experimental method

#### 4.1 Cell Culture.

Human hepatocellular liver carcinoma cell line (HepG2 cell) and human hepatocyte cell line (HL-7702 cell) were obtained from the Committee on Type Culture Collection of Chinese Academy of Sciences. Cells were grown in cell culture media and incubated at 37 °C in a 5% CO<sub>2</sub> humidified incubator (MCO-15 AC, SANYO). The two cell culture medium were DMEM, and RPMI - 1640 supplemented with 10 % heat-inactivated fetal bovine serum (FBS), 1% penicillin-streptomycin.

#### 4.2 MTT Assay.

Cytotoxicity was measured by applying the MTT assay. HepG2 and HL-7702 cells were seeded in a 96 well-plate ( $5 \times 10^4$  cells/well) and incubated for 24 h (37 °C, 5% CO<sub>2</sub>). Then fresh medium containing increasing concentrations of CTMP was added to each well, respectively. After 24 h incubation, medium was removed and replaced with medium containing MTT (0.5 mg/mL). Cells were incubated at 37 °C for another 4 h. DMSO (100  $\mu$ L) was added to lyse the cells and dissolve the formazan produced. The absorbance at 490 nm of each well was monitored using the microplate reader, and the viability was calculated based on the recorded data.

#### 4.3 Low Cytometry (FCM) Analysis

The fluorescence of CTMP treated cells was quantitatively analyzed *via* flow cytometer. Briefly, HepG2 and HL-7702 cells were respectively cultured in the 30 mm culture dish ( $10^5$  cells per dish) for 24 h. By removing the culture medium, each group of cells were added medium containing CTMP (100  $\mu$ g/mL) for 4 h incubation. The cells were collected by centrifugation (1,000 rpm, 3 min) and re-dispersed in PBS buffer (pH = 7.4, 100  $\mu$ L) before transferring into micro-centrifuge tubes for FCM analysis. The images were obtained *via* an excitation wavelength at 488 nm, and analyzed through IDEAS software.

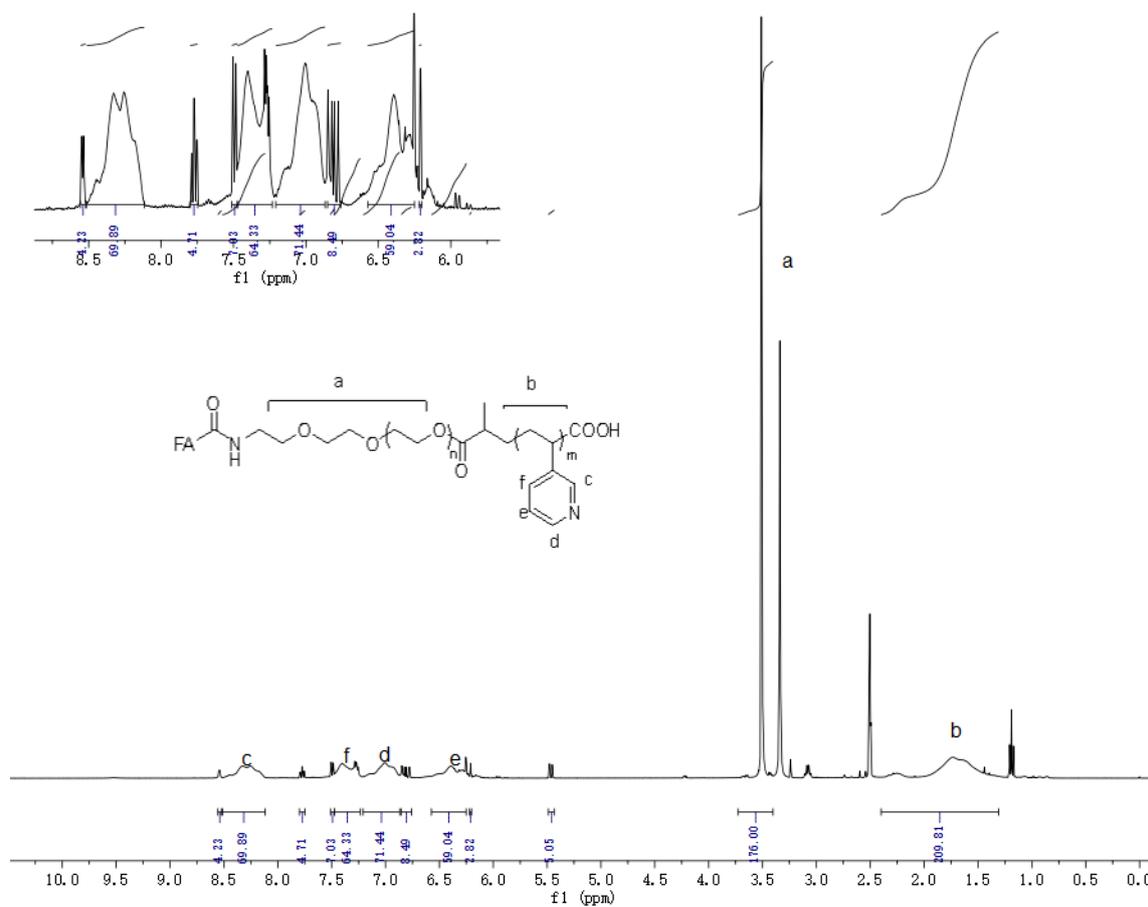
#### 4.4 CTMP-based Nanocarriers for Cancer Therapy *in Vivo*

Male nude mice (6-8 week old, ~20 g) were housed under normal conditions with 12 h light and dark cycles and access to food and water *ad libitum*. Right flank of the mice were injected with a suspension of  $1 \times 10^6$  HepG2 cells in PBS (150  $\mu$ L). When mouse tumors grew up to about 150 mm<sup>3</sup>, they were randomly divided into four groups to receive different treatments. Their body weights and tumor sizes were recorded every 2 days. The four groups were administered for 20 days with 50  $\mu$ L normal saline (NS) as the control, CTMP, free AA, CTMP-AA, respectively (Injection volume for 25 mg/kg).

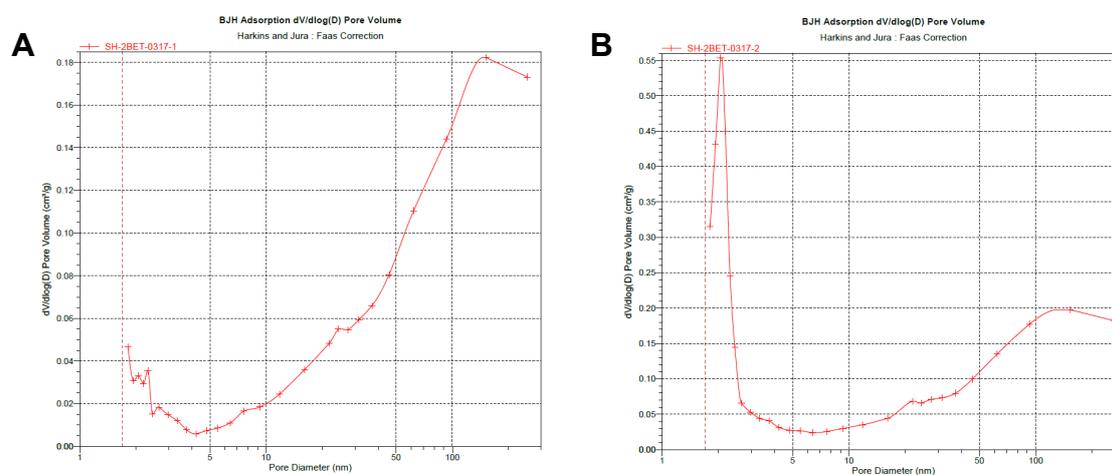
An established equation,  $V = ab^2/2$ , was used to calculate the tumor volume. Herein, V is the tumor volume, while a and b stand for the maximum or minimum diameter of tumor, respectively. Relative tumor volume equals to  $V/V_0$ , where  $V_0$  stands for the initial

tumor volume when the treatment started. Mice were imaged by Caliper IVIS Lumina III *in vivo* fluorescence imaging system. All animal experiments were carried out according to the Principles of Laboratory Animal Care (People's Republic of China) and the Guidelines of the Animal Investigation Committee, Biology Institute of Shandong Academy of Science, China.

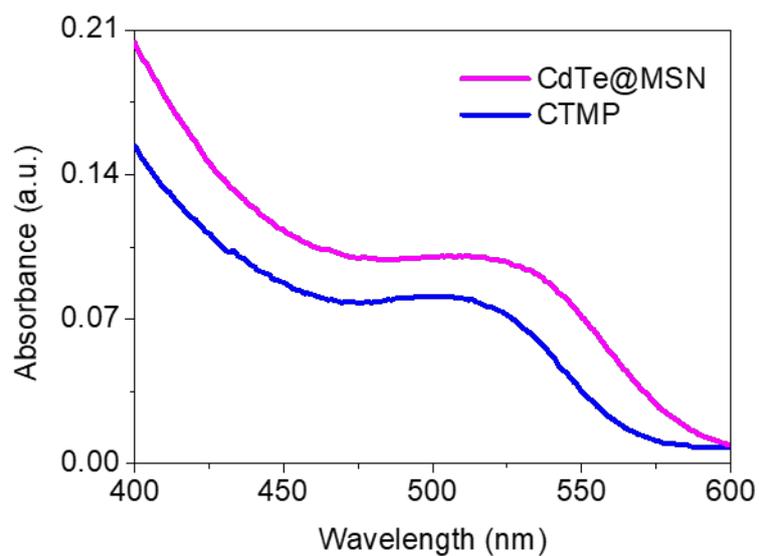
## 5. Supplementary Figures



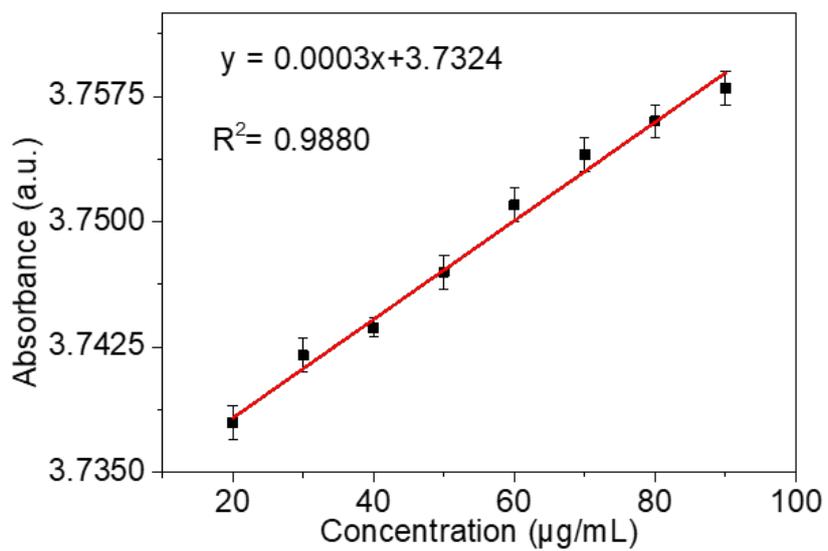
**Figure S1.**  $^1\text{H}$  NMR spectrum of HOOC-PVP-PEG-FA provided by Xin Qiao Biotechnology (Hangzhou, China).



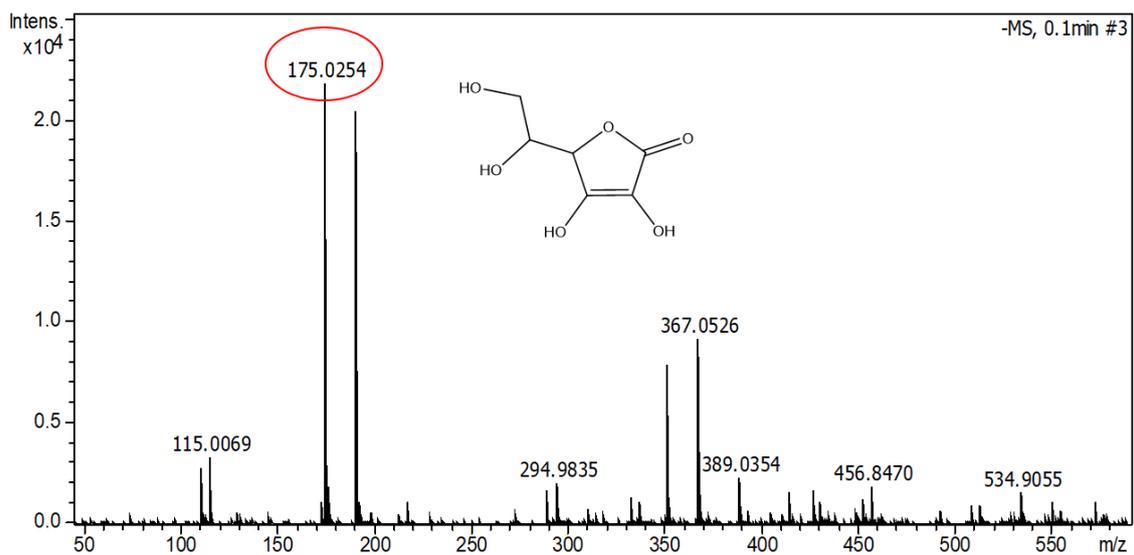
**Figure S2.** The pore diameter distributions of (A) the CdTe@MSN nanoparticles and (B) the CTMP.



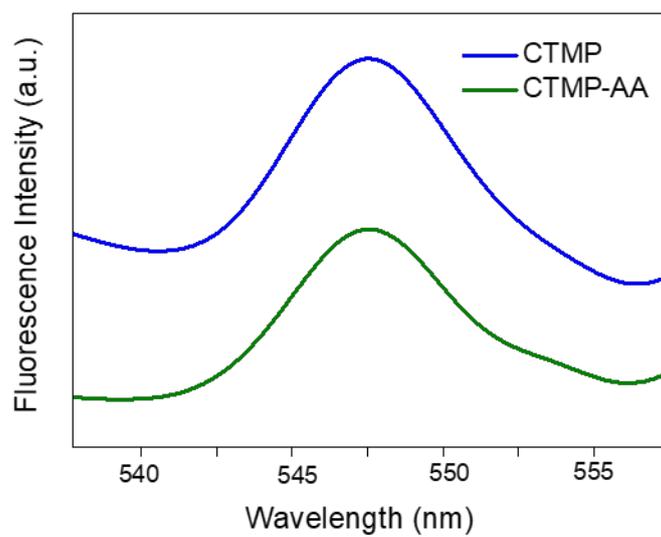
**Figure S3.** UV-Vis absorption spectra of the CdTe@MSN (Pink) and the CTMP (Blue).



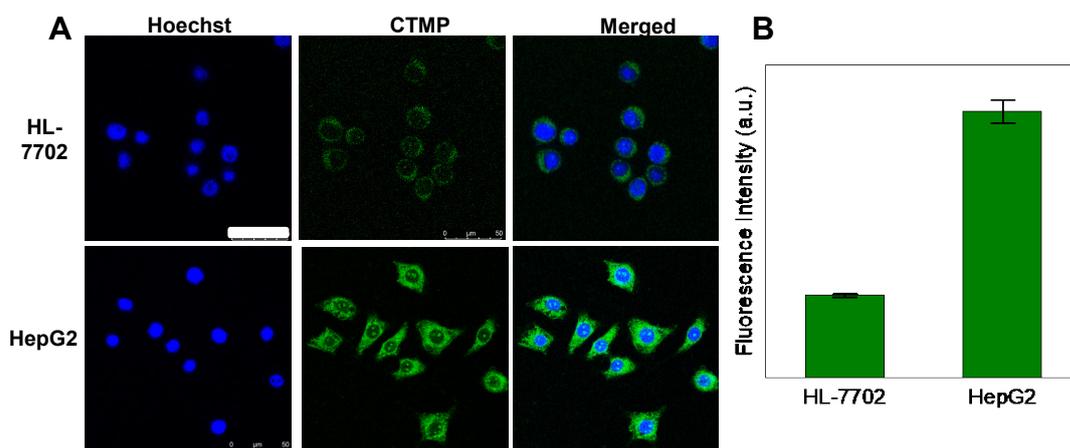
**Figure S4.** A standard linear calibration curve for AA's concentration with absorbance intensities.



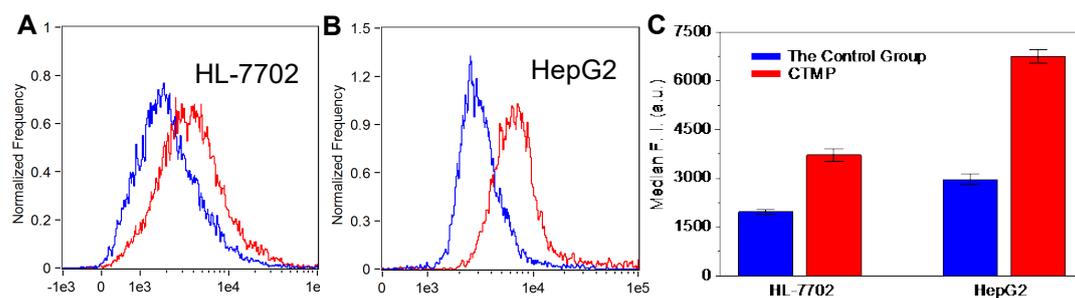
**Figure S5.** HRMS spectrum of ascorbic acid in buffer solution (pH = 6.0) released from CTMP-AA.



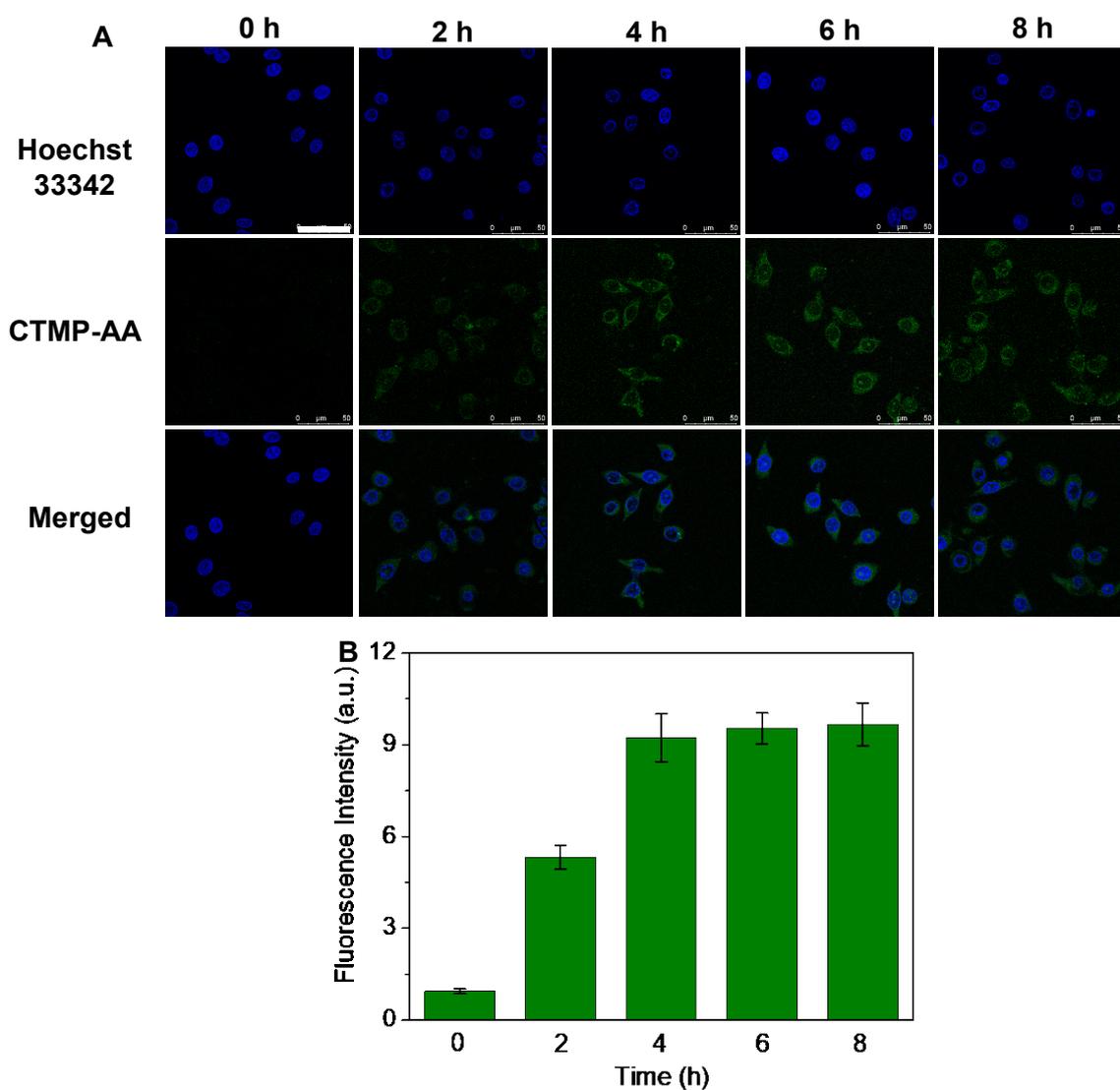
**Figure S6.** Fluorescence intensity change of CTMP after drug loading.



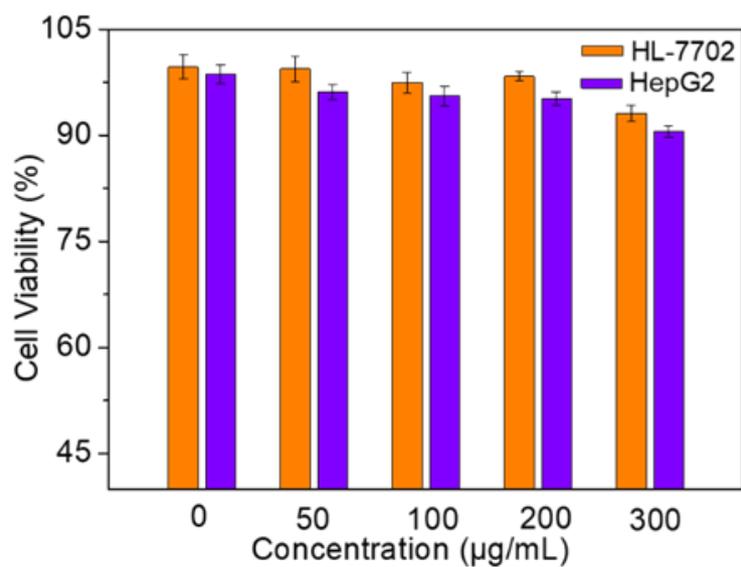
**Figure S7.** (A) The verification of CTMP's targeting ability towards HepG2 and HL-7702 cells by confocal fluorescence Imaging. The nucleus was stained with Hoechst 33342. (B) The quantitative fluorescence intensities of results in (A). Scale bars are 50  $\mu\text{m}$ .



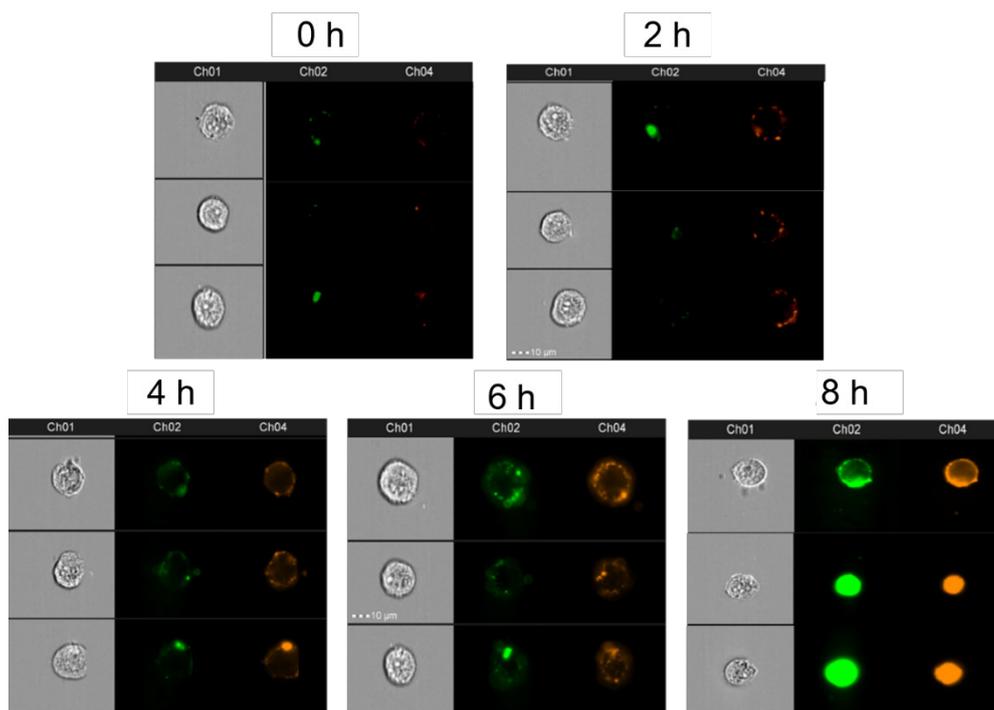
**Figure S8.** The targeted fluorescence images in (A) HL-7702 cells and (B) HepG2 cells obtained by flow cytometry. (C) The quantitative fluorescence intensities in (A) & (B).



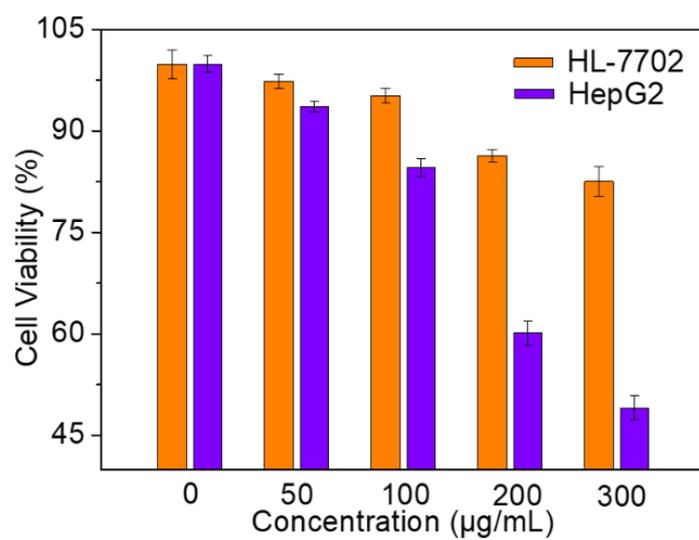
**Figure S9.** (A) Confocal fluorescence images of CTMP-AA in HepG2 cells at different time intervals (0, 2, 4, 6 & 8 h). (B) The quantitative fluorescence intensities of results in (A). Scale bars are 50  $\mu\text{m}$ .



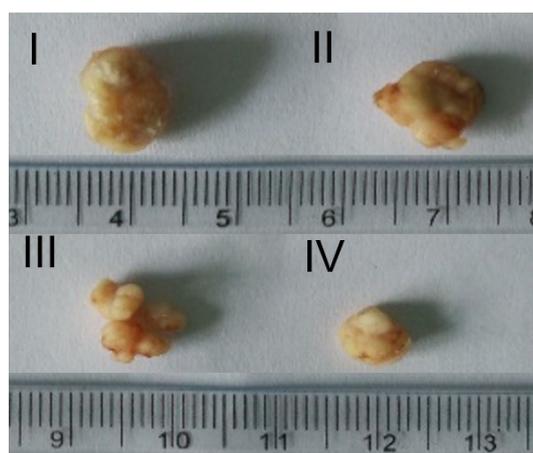
**Figure S10.** Cell viability test at different CTMP's concentrations for HL-7702 cells (orange) and HepG2 cells (blue).



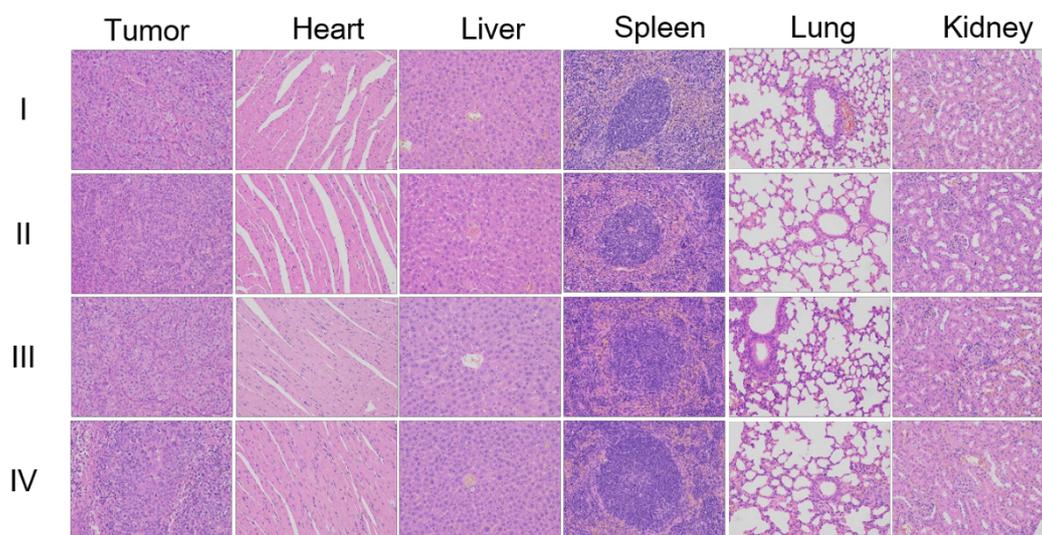
**Figure S11.** The flow cytometry results of apoptotic HepG2 cells assessed by Annexin V-FITC/PI double staining. CTMP-AA incubated with HepG2 for different time intervals (0, 2, 4, 6 and 8 h).



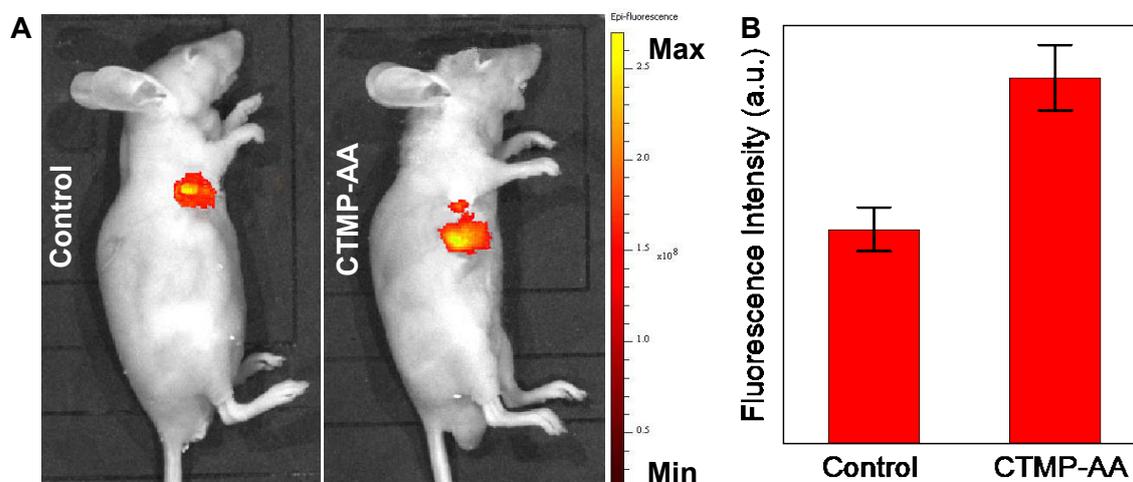
**Figure S12.** Cell viability at different CTMP-AA's concentrations for HL-7702 cells (orange) and HepG2 cells (blue).



**Figure S13.** Pictures of the *ex vivo* tumors of the nude mice for (I) Normal saline (NS) as the control group, (II) Free AA, (III) CTMP and (IV) CTMP-AA.



**Figure S14.** The tumors tissues and organs (heart, liver, spleen, lung and kidney) stained by H&E: (I) NS as the control group, (II) Free AA, (III) CTMP and (IV) CTMP-AA.



**Figure S15.** (A) *In vivo* fluorescence images of HepG2 tumor-bearing mice which treated with CTMP-AA for 20 days, and orthotopically injected with DCI-MQ (10  $\mu$ M, 100  $\mu$ L) for 30 min. (B) Relative fluorescence intensities of (A). ( $\lambda_{ex}$ : 560 nm,  $\lambda_{em}$ : 670 nm)