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Supplementary Information

Folic Acid Modified UiO-66 Nano Drug Carrier for Combination Therapy

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Detection of singlet oxygen in vitro of nano drug carrier

Free RB was used as the control, 7 ml of DPBF (120 μM) solution was added into 7 ml of RB (4 ppm) solution, and the mixture was stirred at room temperature for 15 min to make it fully mixed. Then the reaction was carried out under the irradiation of 552 nm laser, and 1 ml of mixture was taken out at a specific time point for UV detection. The sampling time was 0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 min. The UV spectra measured at different time points are used as UV spectrum curve and dynamic change diagram. The detection method of nanocomposites (RB-CPT@UiO-66-FA, RB-DOX@UiO-66-FA) is the same as that of free RB, except that the concentration is prepared at 100 ppm.

In vitro drug release of nano drug carrier

Nano drug carriers were dispersed in PBS with pH = 7.4 and 5.5 to simulate the pH environment in normal and tumor tissues. First, 5 mg nano drug carrier (RB-Drug@UiO-66-FA) was dispersed in 5 ml of PBS buffer with pH = 5.5 or pH = 7.4, then shaking at 37 ℃ with 100 rpm. Take out 1 ml of solution at different time points (0.5, 1, 2, 4, 6, 8, 12, 24, 48 and 72 h), and measure the absorbance of the load at the characteristic absorption peak with UV-vis spectrometer. Three parallel experiments were conducted in each group.The calculation formula is as follows:

$$Encapsulation\ Efficiency(EE)\% = \frac{Total\ amount\ of\ drug - \ amount\ of\ unbound\ drug}{Total\ amount\ of\ drug}$$

$$Load\ Capacity(LC)\ \% = \frac{Total\ amount\ of\ drug - amount\ of\ unbound\ drug}{Total\ amount\ of\ nanoparticles}$$

Cytotoxicity test

In order to detect the cytotoxicity and therapeutic effect of nanocomposite carrier in vitro, we used CCK-8 kit to detect the cell survival rate.

(1) Cell resuscitation:

① Open the water bath and adjust the temperature to 37 ℃.

② After thawing the cells in the negative bath, shake the cells out of the negative bath quickly.

③ Take a 15ml sterile centrifuge tube, add 7ml medium, and then add the cells in the cryopreservation tube.

④ Centrifuge for 5 min at 1000 rpm at room temperature.

⑤ Take a new 25 T cell culture bottle and add 5 ml of medium containing 10% FBS.

⑥ Discard the supernatant in the centrifuge tube, leave sediment and add it all to the culture bottle.

⑦ Under the condition of 5% CO_2 and 37 ℃, stand for 8 h, then suck away all the medium, add 5 ml of fresh medium containing 10% FBS and continue the culture. The experiment can be carried out when the cells are full of culture bottles.

(2) Effects of different nano drug carriers on HepG2 cells

First, the cells are expressed as 1×10^4 was cultured in 96 well plate for 24 hours under 37 ℃ and 5% CO_2 atmosphere. Add 100 μL of nanocomposite carrier (UiO-66, drug, RB-Drug@UiO-66-FA) with different concentrations (6.25, 12.5, 25, 50, 100 μg/ml) treatment was the experimental group, in which the experimental group was divided into two groups: one group did not do any treatment, and the other group of cells were irradiated with 552 nm laser for 10 min. The cells treated with medium were the control group. The three groups of cells were cultured in 37 ℃ and 5% CO_2 atmosphere for 24 hours. Then the cells were treated with 1 × PBS was washed three times to remove unbound nanocomposites and incubated in cell culture medium for another 24 hours. Then, the combined CCK-8 / DMEM solution (10 μL / 100 μL) was added to each well and cultured for another 1 h under the same culture conditions. The survival rate of the three groups was measured with a parallel absorbance meter at 450 nm. The survival rate of cells in each well is calculated according to the ratio of the absorbance value measured in each well of the experimental group to the absorbance value of the control group. The calculation formula is as follows :

$$Cell\ survival\ rate\ (\%) = \frac{As - Ab}{Ac - Ab} \times 100\%$$

As: light absorption value of the experimental group;

Ac: light absorption value of the control group;

Ab: absorbance value of blank cuvette

Determination of intracellular singlet oxygen ($^1O_2$)

The formation of intracellular singlet oxygen ($^1O_2$) was determined by using 2 ',7 '-dichlorodihydrofluorescein diacetate (DCFH-DA) as probe. First, the cells were cultured in a 24-well plate at a density of 2×10^5 for 24 h, and the culture conditions were consistent with the above. 25 μg/mL nano drug carrier (UiO-66, RB-Drug@UiO-66-FA) was added into the well for further culture for 6 h, then the cells were exposed to 552 nm laser irradiation for 10 min and further culture for 1 h. Then 20 μM DCFH-DA was added for co-incubation for 30 min. After 30 min, the cells were washed with PBS for several times to remove unbound nano drugs. Finally, the cells were examined under a confocal laser microscope (DCFH: Ex = 488 nm).

Laser confocal imaging

The cells were inoculated into petri dishes at a density of 2×10^5 and cultured at 37 ℃ for 24 h. After 24 h, the medium was replaced with a medium containing 12.5 μg /mL nanomaterial (RB-Drug@UiO-66-FA), and cultured at 37 ℃ for 2, 4, 8 and 12 h, respectively. Then, the cells were washed with PBS for three times and fixed with 4% paraformaldehyde fixative for 15 min. 1mL DAPI was added and stained at room temperature for 10-15 min. Then, the cells were washed with PBS for three times to remove unbound dye. Finally, cell fluorescence was collected by confocal laser microscopy (DAPI:Ex = 405 nm; RB: Ex = 488 nm).

Flow cytometry

The cells were seeded into 6-well plates with 2×10^5 cells per well and incubated overnight (12 h) in 2 mL medium per well. After 12 h, the nano-drug carrier (RB-Drug@UiO-66-FA) with a concentration of 12.5 μg /mL was added into the cells. One group was the control group and cultured for 12 h, while the other group was the experimental group. The cells were digested with trypsin and replaced with a new medium containing the nano drug carrier after being mixed and irradiated with a 552

nm laser for 10 min. After that, the cells were cultured for 8 h and collected. The cells were digested and collected with trypsin without EDTA, 500 μL Binding Buffer suspension cells were added, 5 μL Annexin V-FITC was added and mixed, and 5 μL PI was added. After mixed, the cells were detected by machine without light.

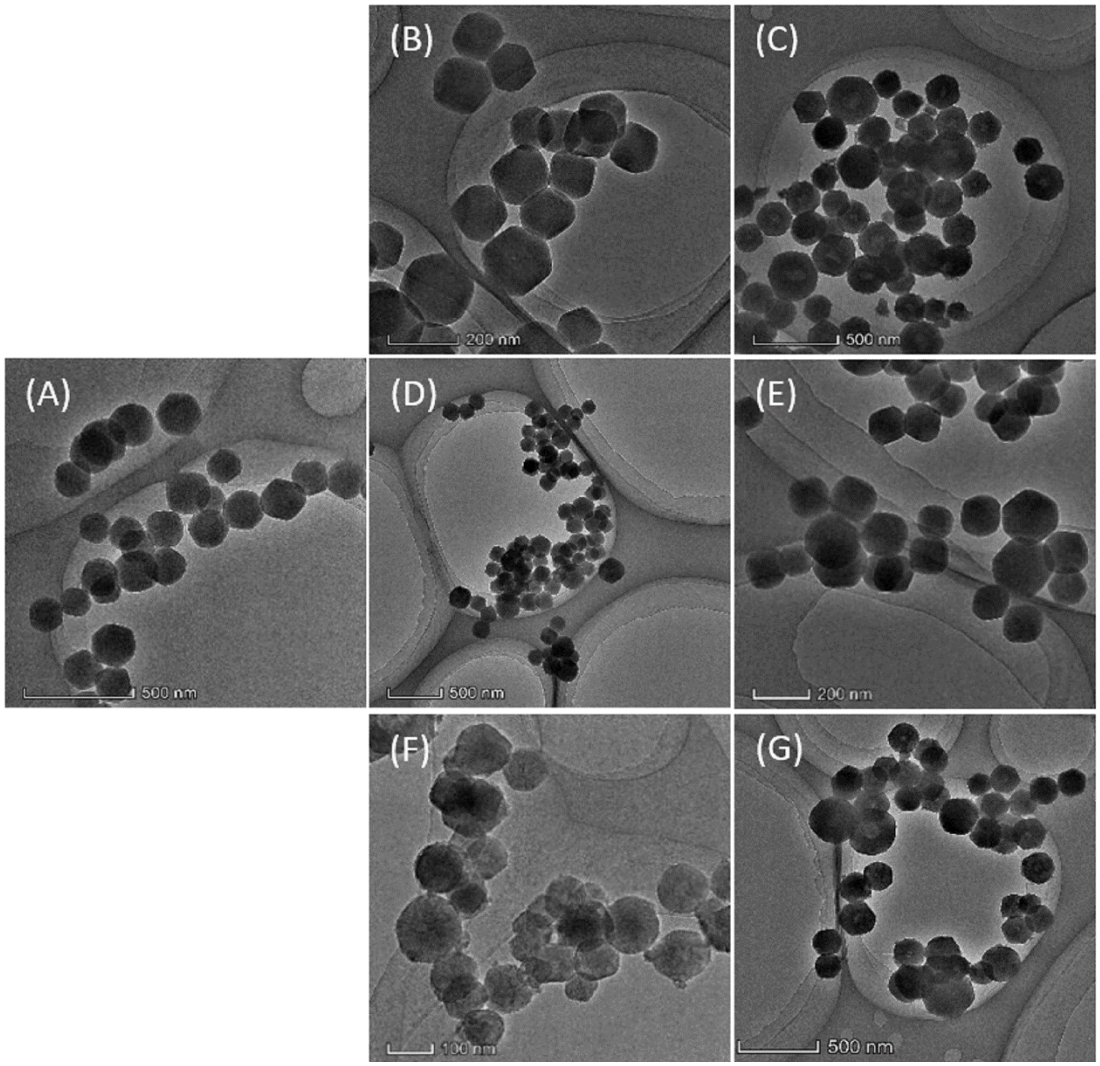

Fig.S1 TEM of (A)UiO-66, (B)CPT@UiO-66, (C)DOX@UiO-66, (D)RB-CPT@UiO-66, (E)RB-DOX@UiO-66, (F)RB-CPT@UiO-66-FA, (G)RB-DOX@UiO-66-FA

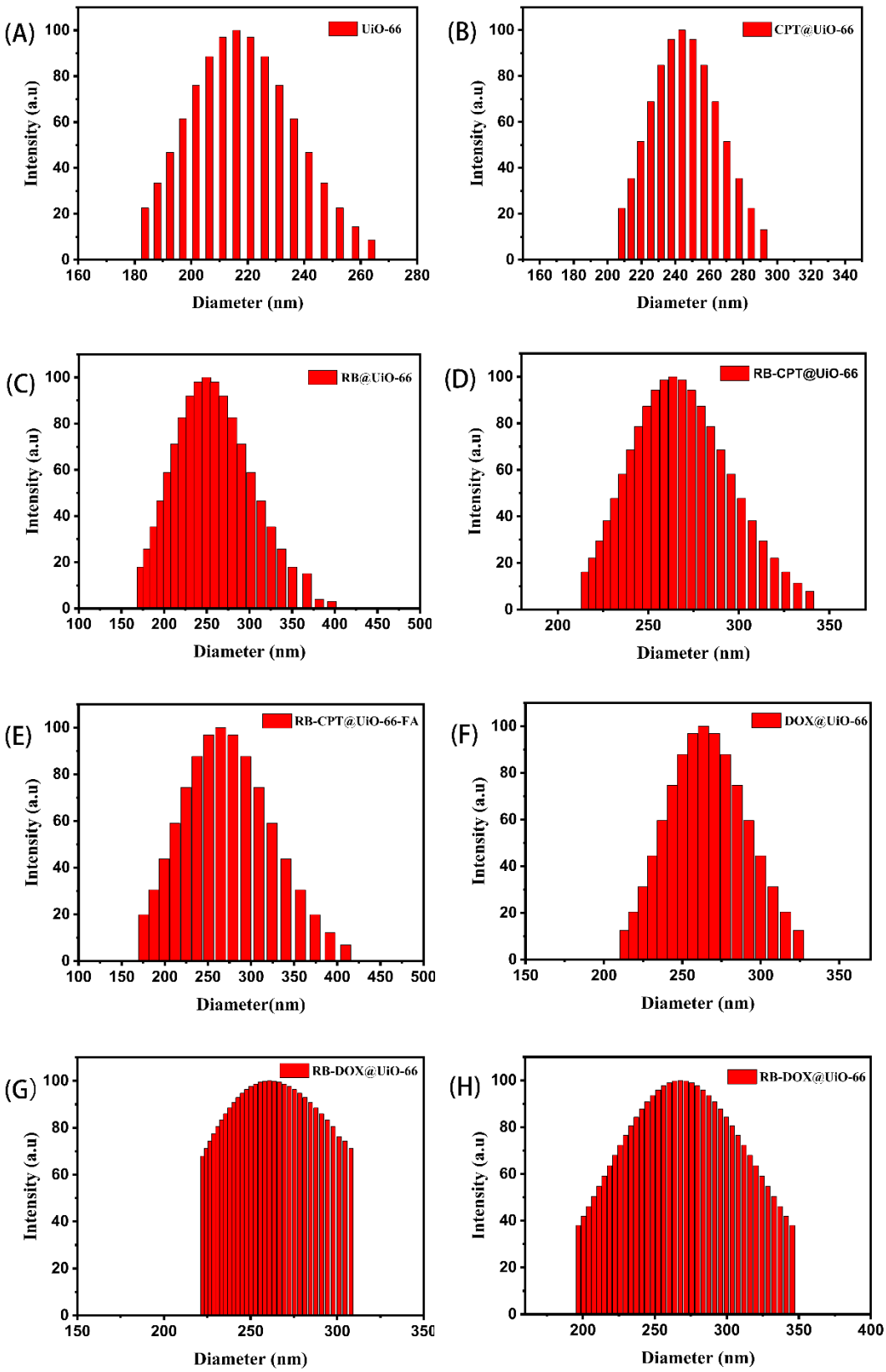

Fig.S2 DLS particle size distribution ((A)UiO-66, (B)CPT@UiO-66, (C)RB@UiO-66, (D)RB-CPT@UiO-66, (E)RB-CPT@UiO-66-FA, (F)DOX@UiO-66, (G)RB-DOX@UiO-66, (H)RB-DOX@UiO-66-FA)

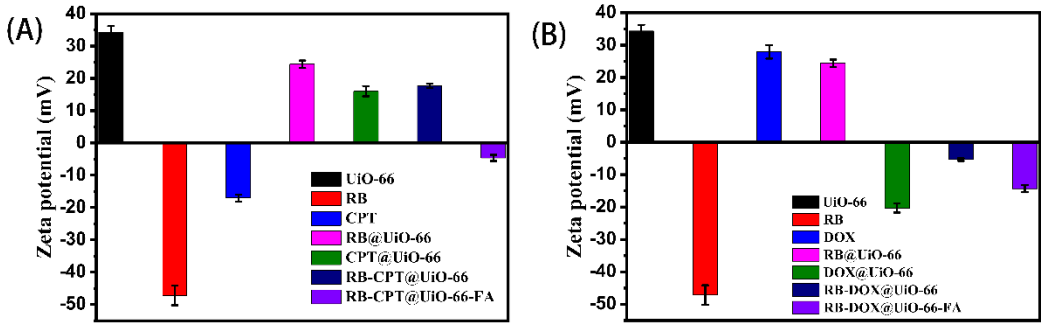

Fig.S3 Zeta potential diagram ((A)Drug=CPT, (B)Drug=DOX)

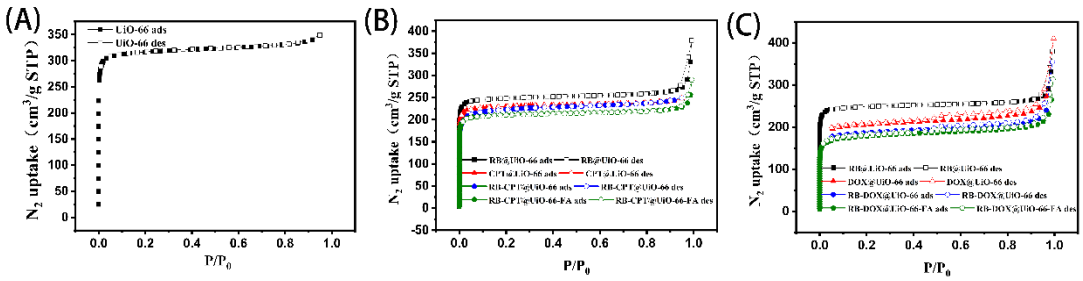

Fig.S4 Nitrogen adsorption diagram ((B) Drug=CPT, (C)Drug= DOX

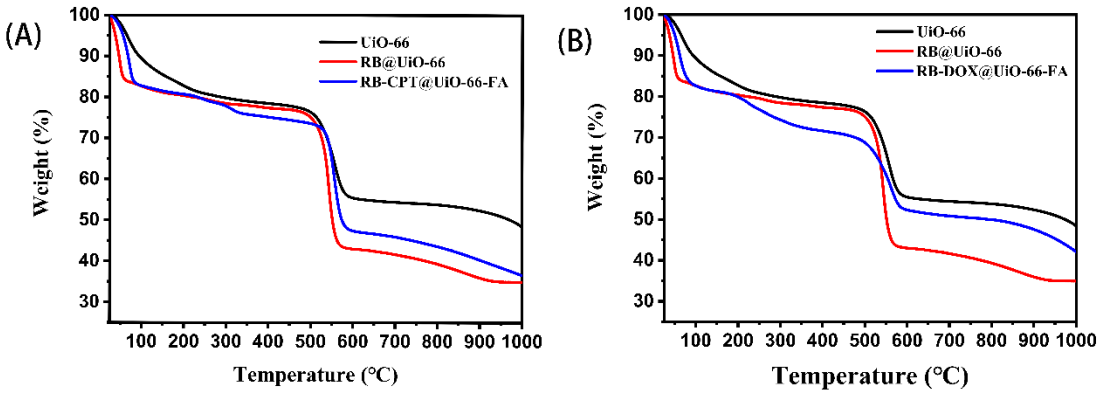

Fig.S5 Thermogravimetric analysis diagram ((A) Drug=CPT，(B)Drug= DOX)

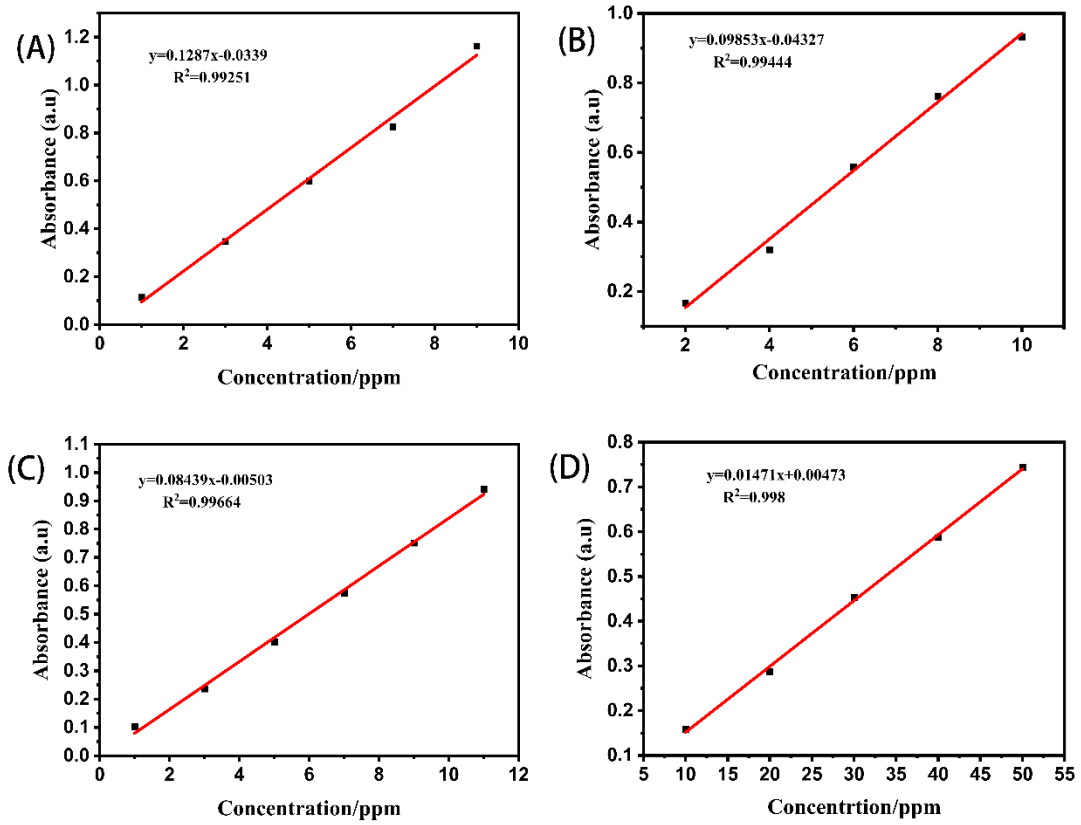

Fig.S6 Standard curves of CPT, DOX, RB ((A) Standard curve of CPT ethanol solution, (B) Standard curve of RB ethanol solution, (C) Standard curve of RB phosphoric acid buffer solution, (D) Standard curve of DOX phosphoric acid buffer solution)

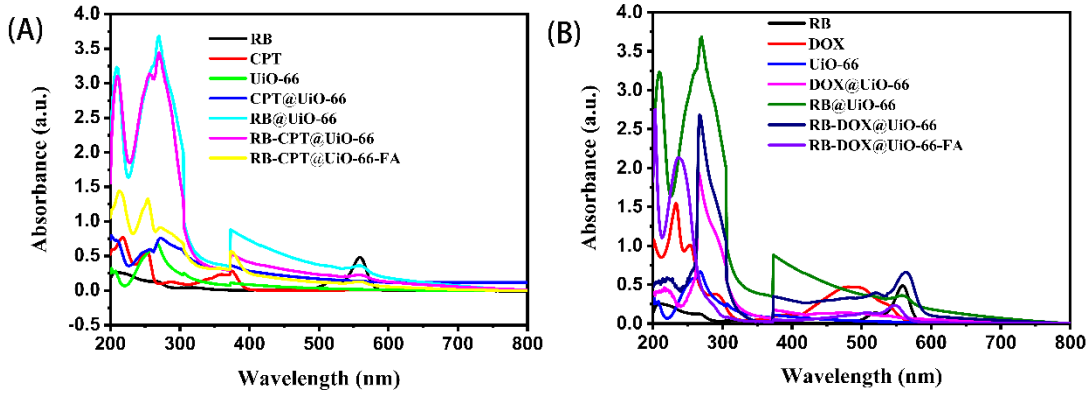

Fig.S7 Ultraviolet absorption spectrum ((A) Drug=CPT，(B)Drug= DOX)

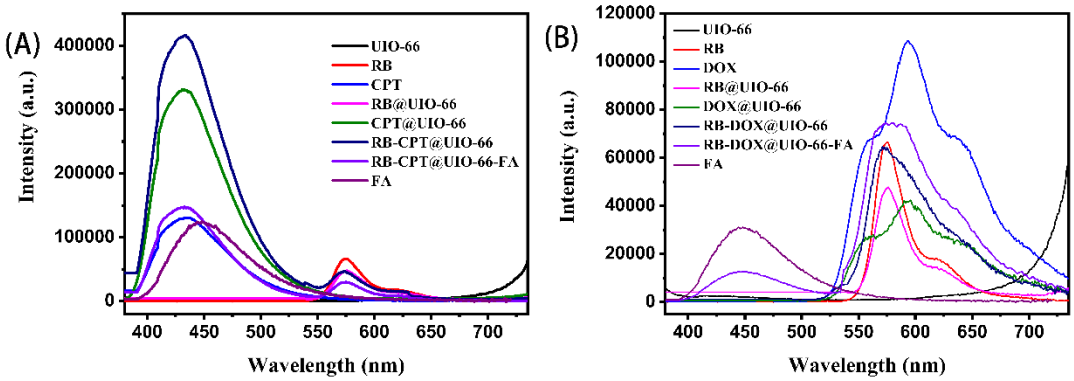

Fig.S8 Fluorescence spectrogram ((A) Drug=CPT，(B)Drug= DOX)

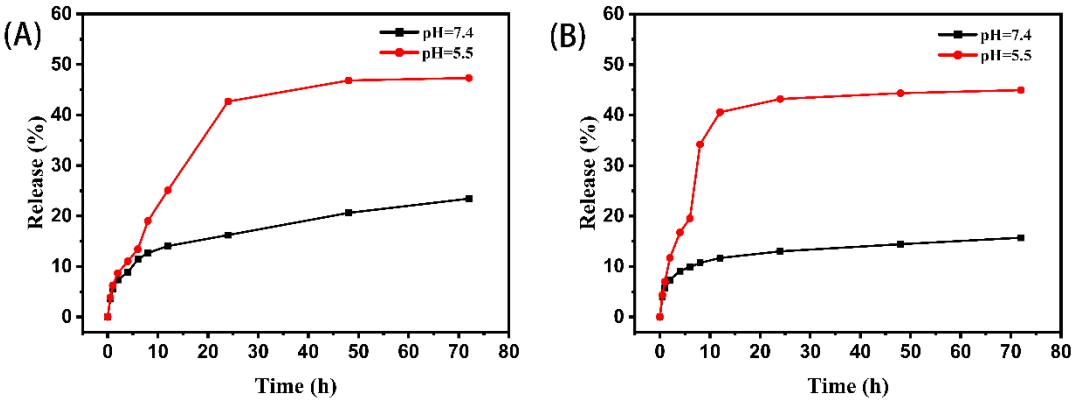

Fig.S9 RB-Drug@UiO-66-FA Drug release in PBS at pH=5.5 and 7.4（Drug (A)=CPT，(B)=DOX）

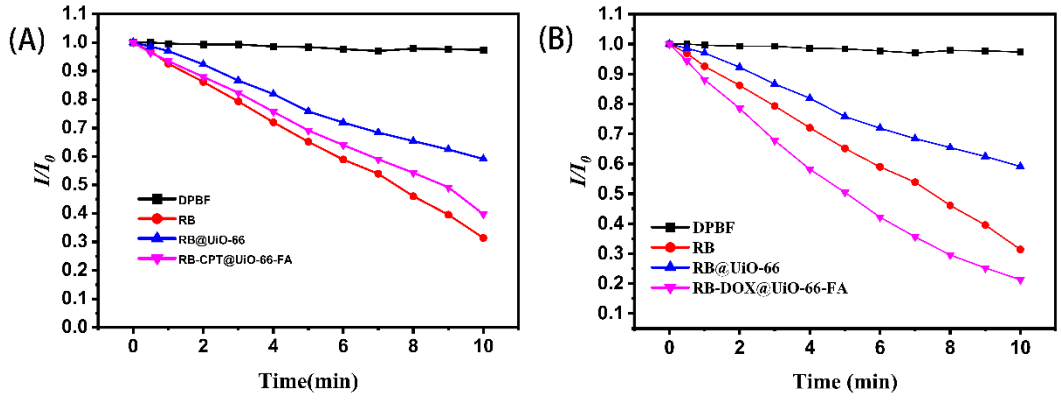

Fig. S10 Kinetic changes of RB, RB@UiO-66, RB-Drug@UiO-66-FA and DPBF (120 uM) DMF solutions irradiated by 552 nm laser ((A) RB-CPT@UiO-66-FA，(B) RB-DOX@UiO-66-FA)