Electronic Supplementary Material (ESI) for Integrative Biology.

Acetate ion enhance load and stability of doxorubicin onto PEGylated nanodiamond for selectively tumor intracellular controlled release and therapy

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Experimental Section

Stability

The stability of ND-PEG-DOX/NaAc with a concentration of 0.5 mg·mL^{-1} in the PBS (pH 7.4) buffer solution was determined at 4 ℃ for a period of up to 450 days. Samples were kept in plastic vials and protected from light. The liquid supernatant of samples was obtained by centrifugation and measured the absorbance at 480 nm of DOX at set intervals. Drug leakage (%) was evaluated as the percentage of free drug in the nanoparticles suspension increased.

The effect of NaAc medium on DOX adsorption

To verify the important role of NaAc medium on DOX adsorption, a PB medium with the pH of 9.87 was used instead of NaAc medium. Specifically, duplicate of ND-PEG (1 mg) were dispersed into the medium of PB (pH 9.87) and NaAc (1.0 M, pH 9.87) to sonicate for 1 h at room temperature followed by adding DOX (200 μg) and mixed thoroughly, respectively. After shaking for 6 h, the solution was centrifuged to remove any non-adsorbed DOX. The product was obtained

by washing three times with deionized water and finally placed in a vacuum drying oven and protected from light. The amount of DOX adsorbed was determined by calculating the change in DOX concentration before and after the adsorption using a UV-vis spectrophotometer at 480 nm. The amount of DOX loading on ND-PEG in NaAc medium is 195.0± 1.9 $\mu g \cdot mg^{-1}$, while it is 90.4 ± 2.5 $\mu g \cdot mg^{-1}$ in the PB medium (pH 9.87). So the result demonstrated that NaAc medium played an important role in the adsorption of DOX onto PEGylated nanodiamond, which led to high drug loading of NPDA.

Cell morphology

The effect of ND-PEG, NPDA and free DOX to the morphology of HepG2, HeLa and MCF-7 cells were investigated by optical microscope. Firstly, three kinds of cells were plated into 35 mm cell culture dishes and incubated overnight. Then the culture medium was replaced with fresh medium containing the above mentioned nanoparticles or drug and further cultured for 48 h and 72 h, respectively. Cells were analyzed with optical microscope and the images were captured for analysis.

Cell uptake of time-dependent by flow cytometer

MCF-7 cells (2×10^5) were seeded in 35 mm culture dishes and incubated overnight. The cells were treated with NPDA (5 $\mu g \cdot mL^{-1}$ of DOX equivalent) for 1 h, 3 h, 5 h, 7 h and 16 h, respectively. Then the cells were harvested with trypsin and PBS. The samples were analyzed by flow cytometer. The fluorescence from the NPDA was excited at a wavelength of 488 nm and the emission was collected in the red light signal range. The fluorescence intensity was quantified by Cell Quest software.

Cell uptake mechanism analysis

Role of endocytosis in the uptake and the effect of temperature and cellular energy on the internalization of NPDA by MCF-7 cells were evaluated through the following experiments: Firstly, MCF-7 cells were cultured with NPDA for 3 h either at 37 or 4 ℃. Another group of cells were cultured at 37 ℃ after pretreated with sodium azide (NaN_3, 0.8 M) for 30 min. Second, two pharmacological inhibitors, including Methyl-beta-cyclodextrin (M-β-CD, 0.01 M) and sucrose (0.45 M) were utilized to treat MCF-7 cells for 30 min before incubation with NPDA. After culture for another 3 h, the excessive nanoparticles were removed by washing three times with PBS (pH 7.4) and the cellular uptake of NPDA was analyzed via a flow cytometer.

Figures:

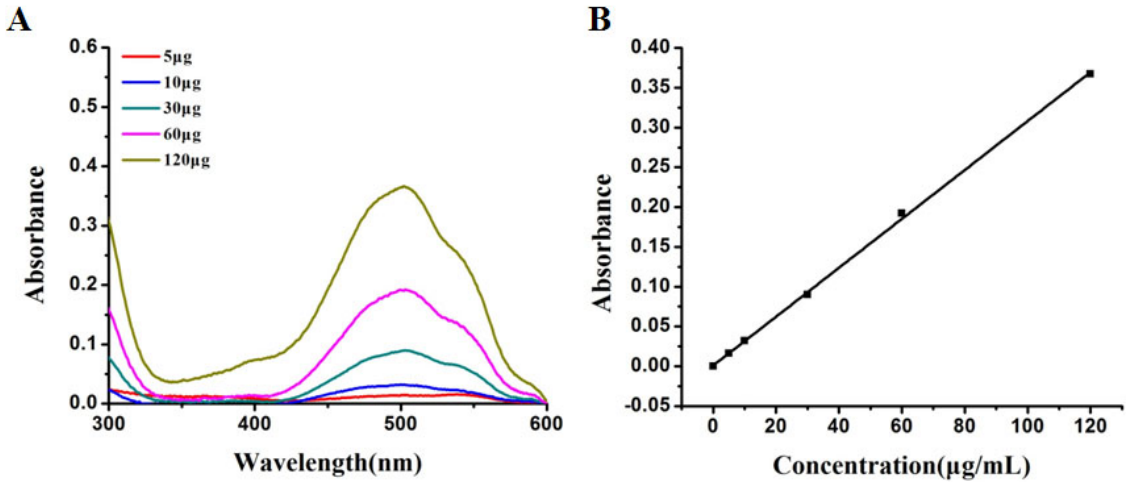


Fig. S1 Standard curve of DOX (A) UV–vis spectra of DOX dilution curves. (B) Linear regression of absorbance versus concentration

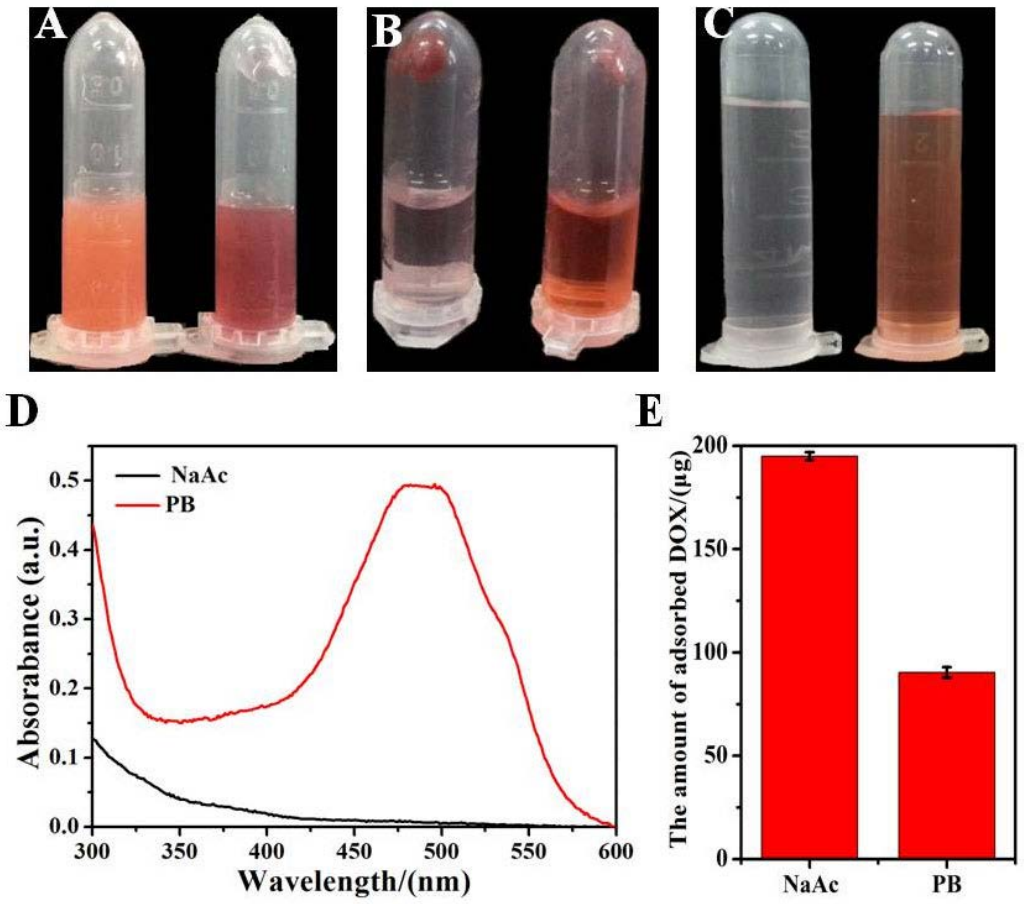


Fig. S2 The effect of NaAc medium on DOX adsorption. (A) The initially mixture solution of ND-PEG and DOX in NaAc medium (right) and PB medium (pH 9.87, left). (B) The images of ND-PEG and DOX in NaAc medium (left) and PB medium (pH 9.87, right) after shaking for 6 h and centrifuging for 5 min. (C) The supernatant after centrifuging and washing for thrice with deionized water in NaAc (left) and PB medium (pH 9.87, right). (D) UV–vis spectra corresponding to (C). (E) The loading amount of DOX on ND-PEG carriers, where one is in the NaAc medium, the other is in the PB (pH 9.87) medium.

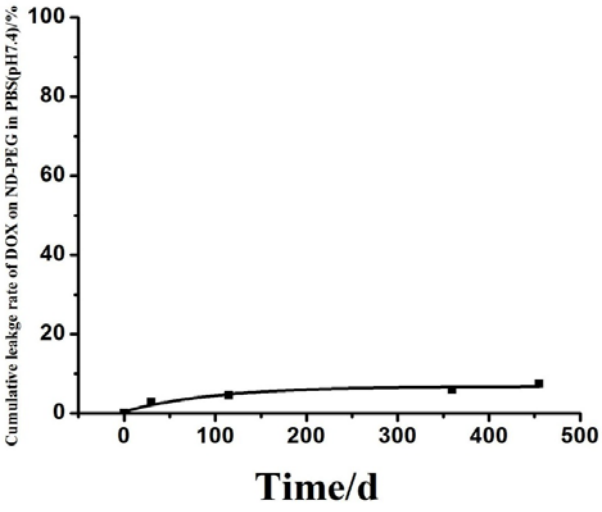


Fig. S3 The stability of NPDA in the PBS (pH 7.4) buffer solution

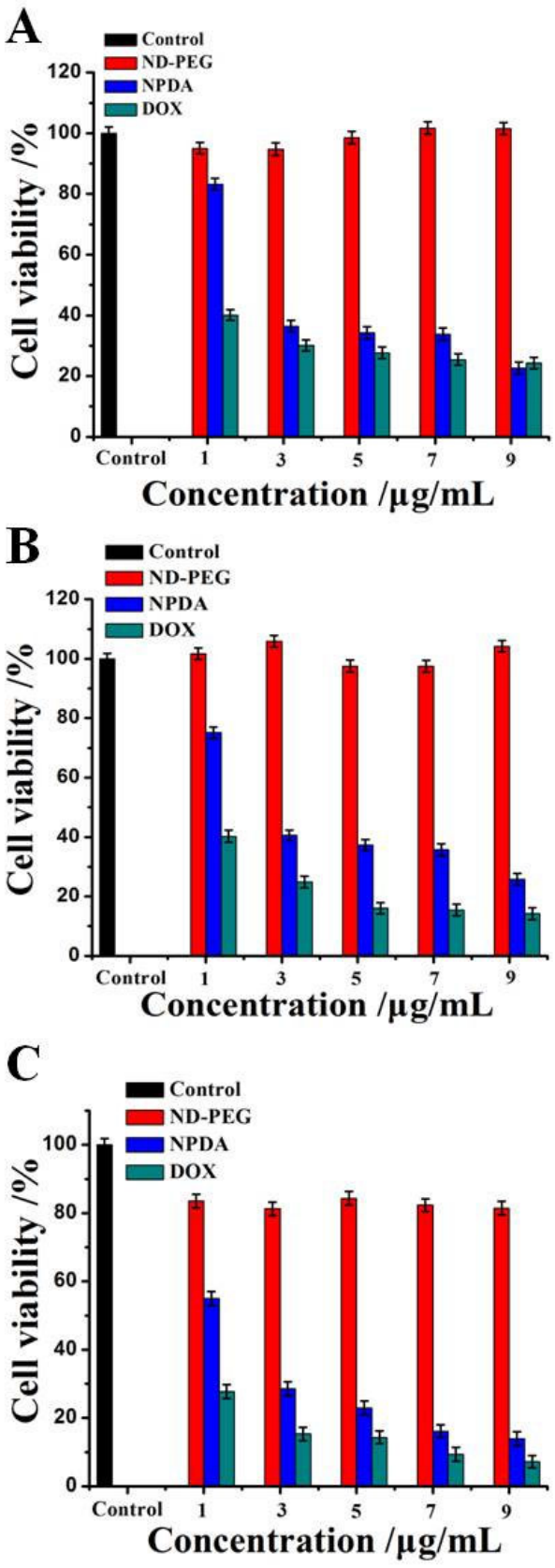
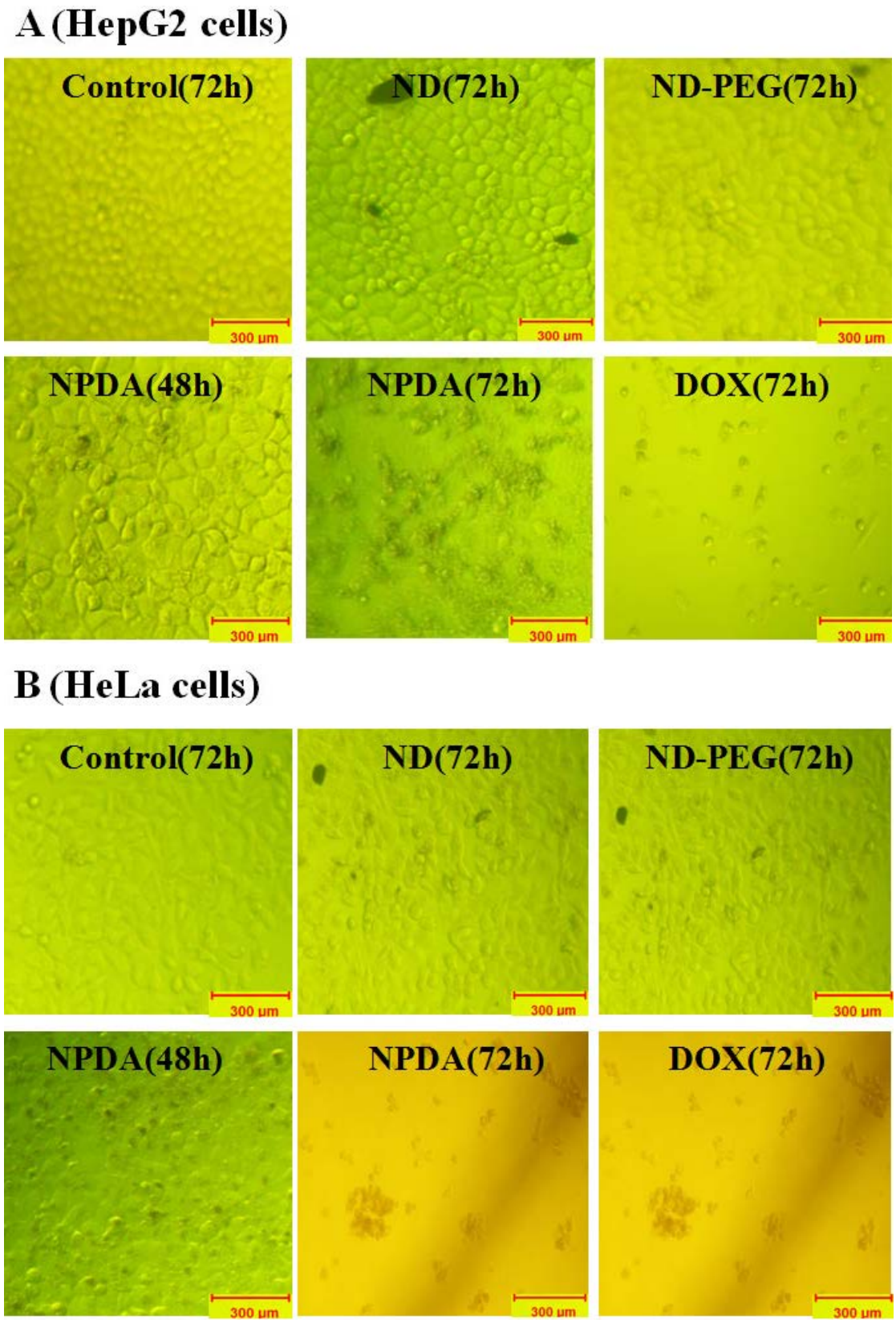


Fig. S4 Effect of free DOX and NPDA on HepG2, HeLa and MCF-7 cells viability for 72 h was measured by MTT assay. (A) HepG2 cells, (B) HeLa cells and (C) MCF-7 cells. Experiments were repeated three times and data are presented as the mean ± SD (for each group, n = 6).


A (HepG2 cells)
Control(72h)
ND(72h)
ND-PEG(72h)
NPDA(48h)
NPDA(72h)
DOX(72h)
B (HeLa cells)
Control(72h)
ND(72h)
ND-PEG(72h)
NPDA(48h)
NPDA(72h)
DOX(72h)
300 μm

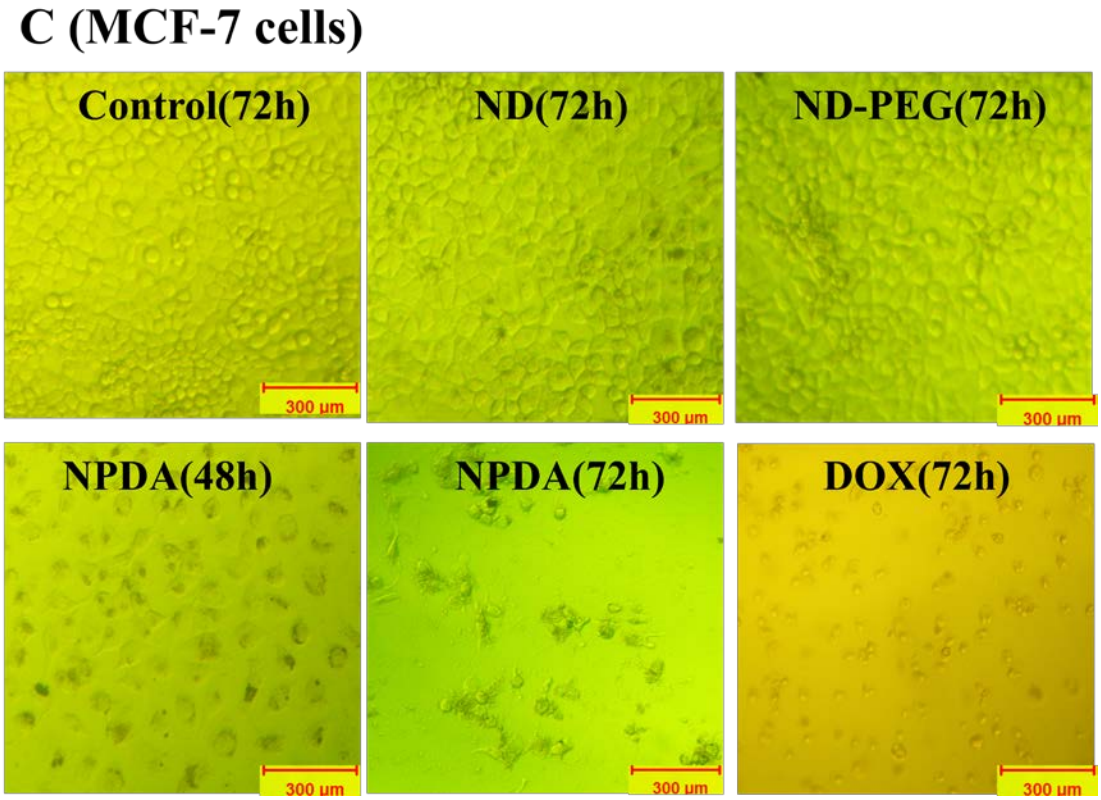


Fig. S5 Microscopy images of cells incubated with nanoparticles for 48 h and 72 h ((A) HepG2, (B) HeLa and (C) MCF-7). scale bar = 300 μm

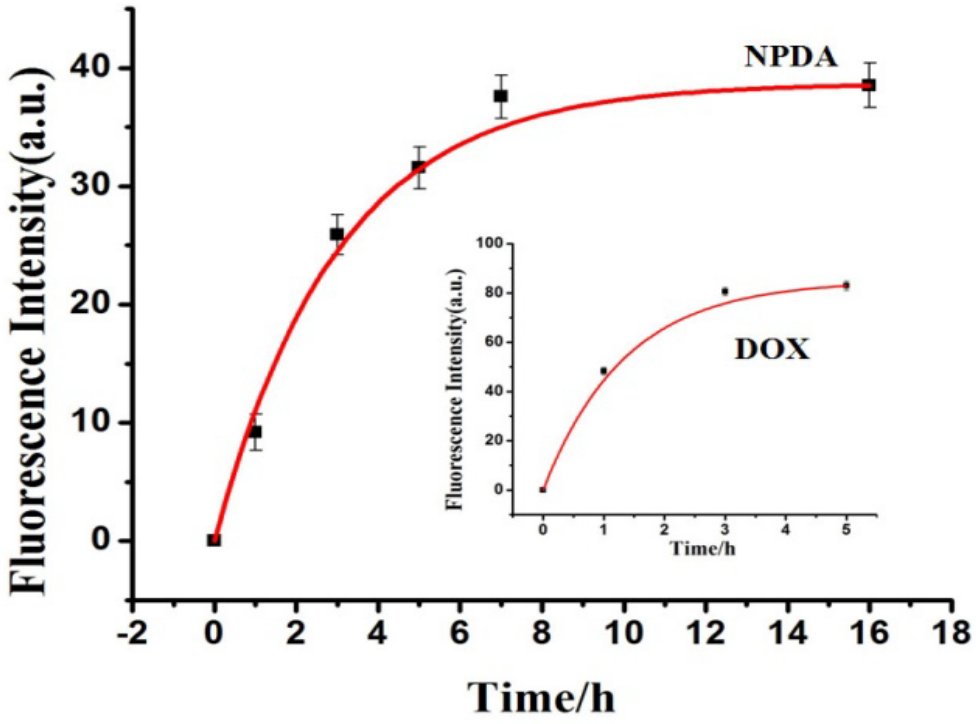


Fig. S6 The kinetics of the cellular uptake of NPDA and free DOX

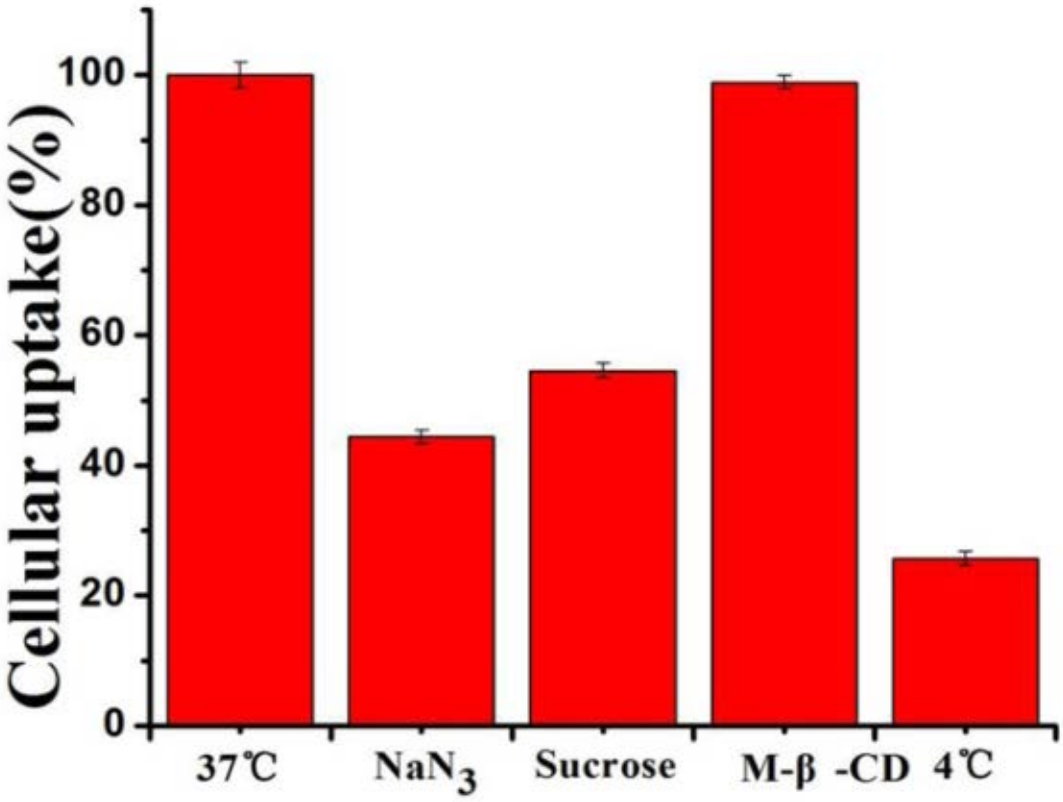


Fig. S7 Quantitative analysis on the uptake mechanism of NPDA nanoparticles by MCF-7 cells.