Supporting Information

Mineralization of pH sensitive Doxorubicin Prodrug in ZIF-8 to Enable the Target Delivery to Solid Tumors

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Experimental Section

Cell culture and maintenance. Triple-negative breast cancer cell line MDA-MB-231 was grown in DMEM medium with 10% FBS and 5 μg/mL insulin at 37 °C and healthy cell MCF-10A was cultured in Eagle's Minimum Essential Medium containing 1% penicillin, streptomycin and epidermal growth factor EGF (20ng/ml final in medium) and 2 mM L-glutamine, in a humidified incubator with 5% CO2. Cells were passaged 2–3 times a week once they reached 90–100% confluency. Breast cancer MDA-MB-231 cells and human normal breast MCF-10A cells were kindly provided by Prof. Jukka Westermarck (Turku Bioscience Centre of Univ. of Turku and Åbo Akademi University, Turku, Finland).

CLSM Imaging. Evaluations of drug and nanoparticle localization in cells were determined by confocal microscopy. Cells were grown on Confocal Dishes (15 × 104 cells per Dish, VWR® 35 mm Confocal Dish, Sterile) overnight. The medium was replaced by solutions of DOX, CAD, CAD@ZIF-8, CAD@ZIF-8-FA, respectively. After 2 h, 16 h time points, the cells were rinsed with PBS twice, fixed with 4% PFA for 5 mins and the sample was dyed by DAPI for 10 mins. A Zeiss LSM780 confocal microscope (Plan-Apochromat 100×/1.40 Oil DIC), oil objective and Zen 2010 software setup were used for imaging. Detection of DAPI was performed with 405 nm laser excitation and 450–500 nm emission. Argon laser 488 excitation was utilized for doxorubicin and emission was collected at 530–600 nm. (the concentration of DOX equal to 4 μg mL^{-1}, n=6).

Flow Cytometry Assay. Cells were incubated overnight for attachment in 6 well plates (1×10^5 cells per well). DOX, CAD, CAD@ZIF-8, CAD@ZIF-8-FA were incubated with cells to keep the final concentration of DOX at 2.5μg/ml in cell media for 1 h and 8 h timepoints at 37 °C. Cells were collected by trypsin, washed twice with

PBS, and acquisition of cellular uptake was determined by a flow cytometer BD LSRFortessa (BD Biosciences) by using the PE channel, the results were analyzed by Flowjo_V10 The gate was defined for live cells only; 10000 cells were recorded per sample.

2. Figures

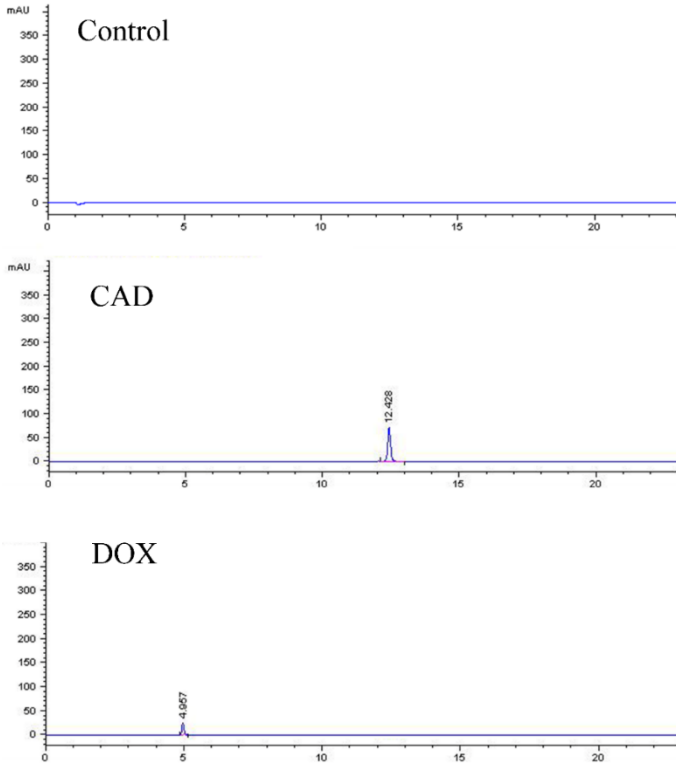

Figure S1. HPLC characterization of CAD and DOX.

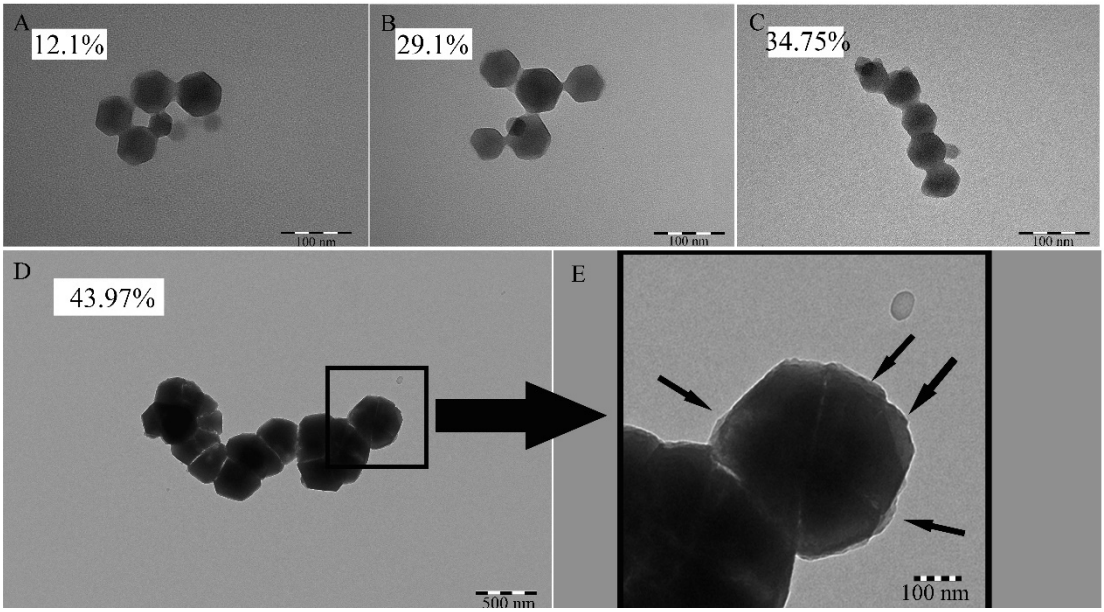

Figure S2. The TEM image of ZIF-8 with different loading degree: (A):12.1%, (B):29.1%, (C):34.75%, (D):43.97%) of CAD. (E) is the magnification of (D) and the CAD layer was marked by arrows.

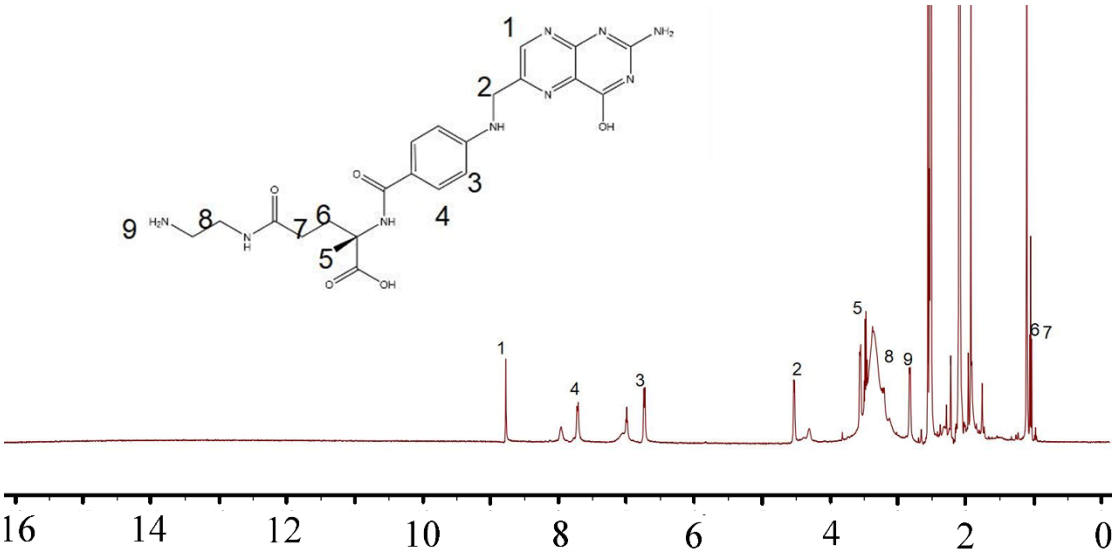

Figure S3. The NMR results of FA-NH_2.

In order to investigate the reasons for the enhanced toxicity of ZIF-8 to DOX at high concentration, we chose DOX concentration of 5 μg mL^{-1} and performed the WST-1 assay with Zn^{2+} ion, ZIF-8, DOX, DOX + Zn^{2+} and DOX + ZIF-8 groups **(Figure S4)**.

The results showed that zinc ions did not increase the toxicity of DOX. Besides, we found that high concentrations of blank ZIF-8 NPs could express toxicity **(Figure 3B and Figure S4)**. This may because the ZIF-8 NPs and CAD@ZIF NPs (34.75% loading degree) showed positive charge (21.5 ± 0.77 mV and 5.2 ± 0.46 mV) and can be easily uptake by the cells with negative cell membrane, while after the FA decorated, the CAD@ZIF-8-FA showed high negative charge (-10 ± 1.03 mV) **(Figure S5)**, hence the unspecific uptake was eliminated. Furthermore, the FA can enhance the tumor folate receptor-mediated uptake, therefore CAD@ZIF-8-FA only caused high toxicity to tumor cells.

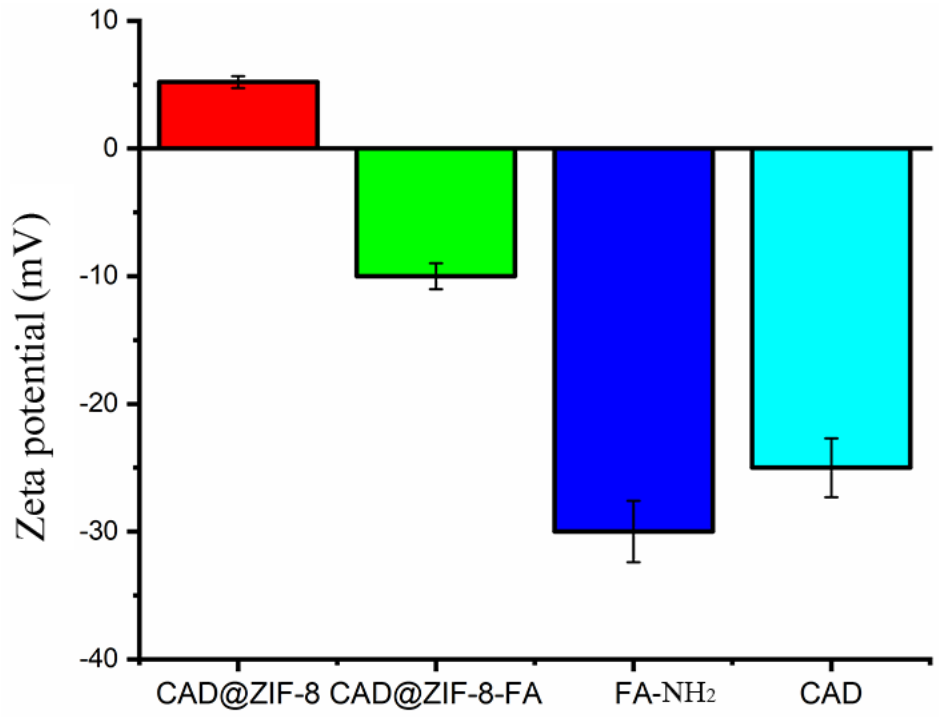

Figure S4. The Zeta potential of CAD@ZIF-8, CAD@ZIF-8-FA, FA and CAD. (n=3)

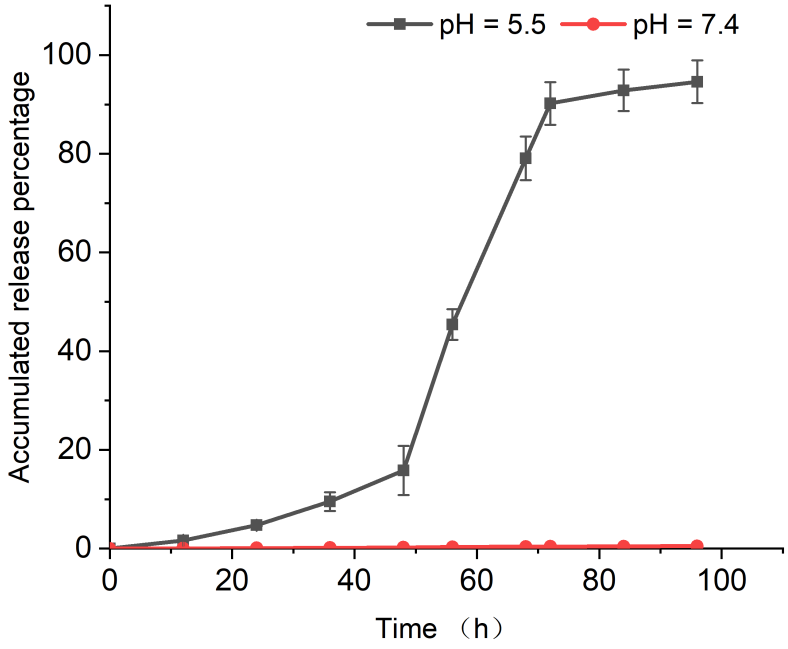

Figure S5. The in vitro release of DOX from CAD@ZIF-8-FA (loading degree equal to 13.5%, n=3).

Figure S5 shows the pH-responsive cumulative release profiles of CAD@ZIF-8-FA. There were negligible releases of DOX from CAD@ZIF-8-FA even after 100 hours at pH 7.4. However, at pH 6.0, more than 95% of DOX released within 100 hours. These results suggested the great acidic pH controlled DOX release of CAD@ZIF-8-FA NPs. Meantime, in the first 50 hours, DOX release was very low (< 20%), which may attribute to the slow breakage of the pH-sensitive linker between “CAD-FA” structure on the surface of ZIF-8.

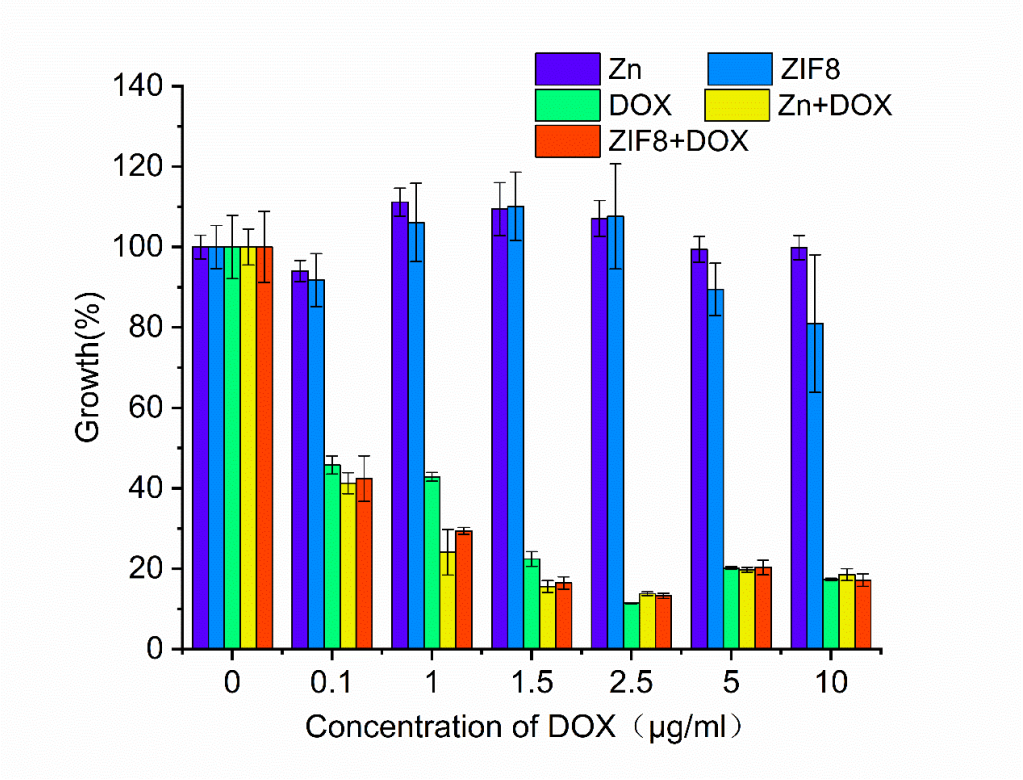

Figure S6. Cytotoxicity of Zn ions, blank ZIF-8, DOX, Zn ions with DOX and ZIF-8 plus DOX for MDA-MB-231. The concentration of different regent corresponds to the content within CAD loaded NPs CAD@ZIF-8@CAD-FA. (n=6)

Since the fluorescent behavior of CAD and DOX in the presence of cells is unknown. We conducted a CLSM experiment to detected whether the PE channel for DOX detection is also good for detecting CAD. First, in the control group, CAD@ZIF-8-FA NPs were incubated with cancer cells for 2 hours before fluorescence imaging. In the experimental group, after incubating CAD@ZIF-8-FA NPs with cancer cells for 2 hours, the medium was replaced with fresh medium, and the incubation continued for another 4 hours before fluorescence imaging was performed. If the excitative wavelength of CAD was different from DOX, then the fluorescence intensity at PE

channel should be different between control and experimental groups. Because the 4 hours of additional incubation will lead to CAD degradation into DOX. As a result, we found that the fluorescence intensity of the experimental group has negligible change **(Figure S7),** suggesting that the PE channel for DOX detection is also good for detecting CAD.

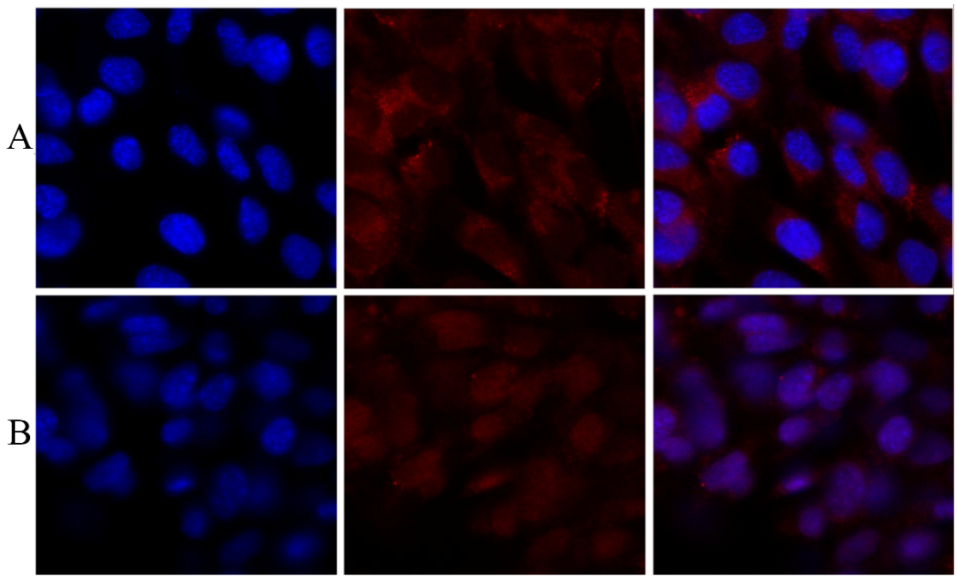

Figure S7. The CLSM results of after incubate the CAD@ZIF-8@CAD-FA NPs with MDA-MB-231 tumor cell. (A) Cells were incubated with NPs for 2h. (B) Cells were incubated with NPs for 2 h then washed with fresh medium and incubate for another 4 h (equivalent to a concentration of DOX of 10 μg mL^{-1}).

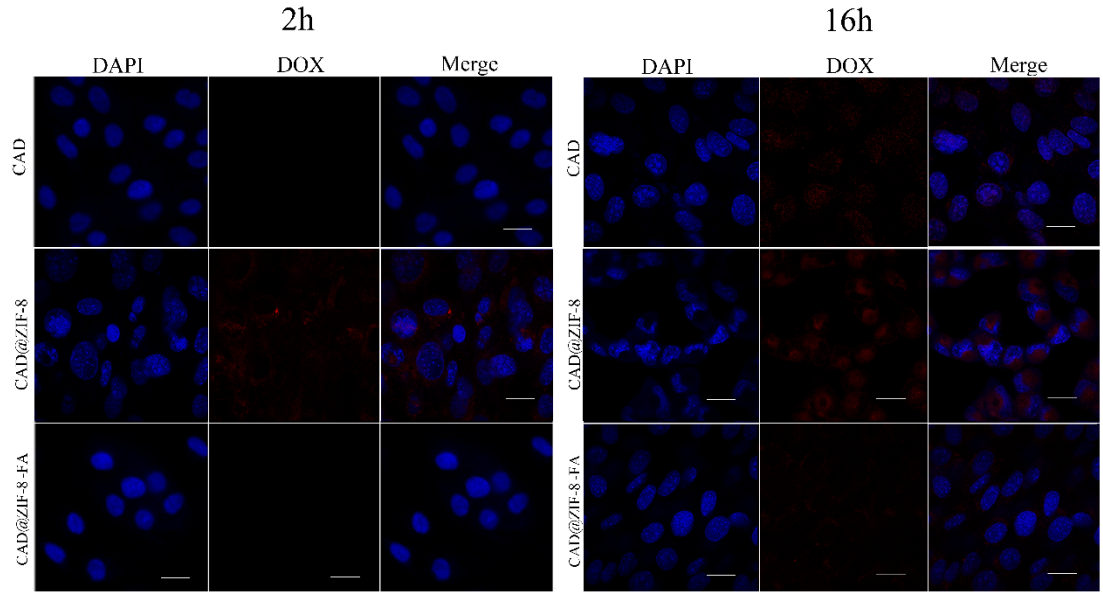

Figure S8. The confocal microscopy images of cellular uptake of CAD, CAD@ZIF-8 and CAD@ZIF-8-FA of MCF-10A. Scale bars denote 20 μm. (Equivalent to a concentration of DOX of 4 μg mL^{-1}).

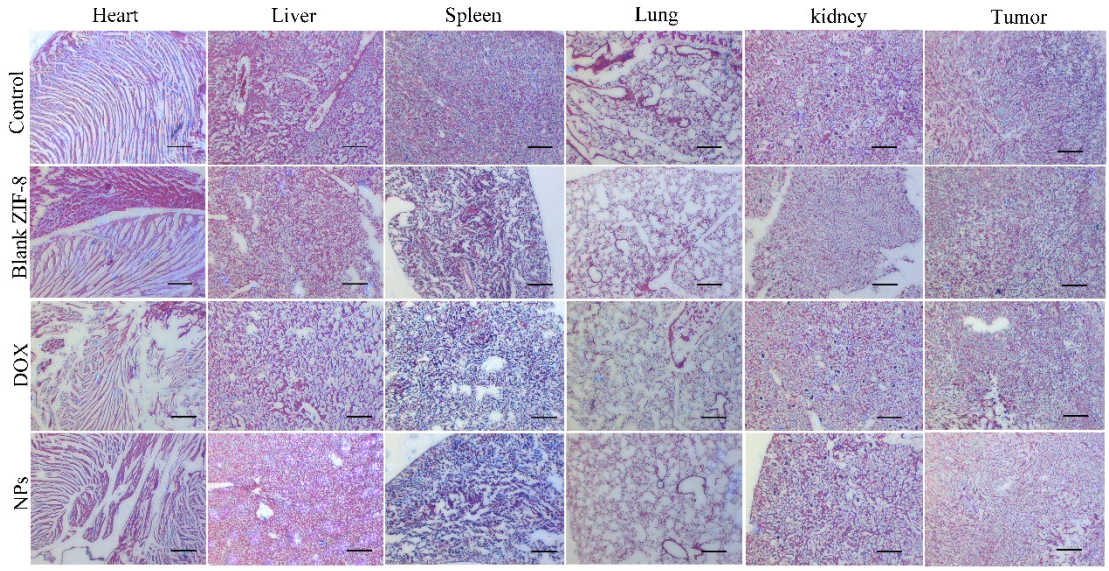

Figure S9. HE stained images of tumors and major organs after treatment with free DOX and CAD@ZIf-8@CAD-FA NPs. (Tissue section in picture magnified 4x) The scale bar was 200 μm.