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**Supplementary Information for**

# Layered double hydroxides bio-composites toward Excellent Systematic Anticancer Therapy

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## Experimental Section

### Materials

Zinc phthalocyanine (ZnPc, 97%), disodium salt of 9,10-anthracenedipropionic acid (ADPA) and doxorubicin hydrochloride (DOX) were purchased from Sigma Aldrich.

Dulbecco's modified Eagles medium (DMEM) containing high glucose, *L*-glutamine and sodium pyruvate, pen-Strep (10,000 units/mL penicillin, 10,000 μg/mL streptomycin) and 0.25% trypsin-EDTA were purchased from Gibco (Invitrogen, Carlsbad, CA). Fetal bovine serum (FBS) was purchased from Hyclone (Thermo Scientific, Rockford, IL). Phosphate-buffered saline (PBS) was purchased from Solarbio Science & Technology Co, Ltd (Beijing, China). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and potassium iodide (PI) were purchased from Sigma. KB cells were obtained from the Institute of Basic Medical Sciences Chinese Academy of Medical Sciences and cultured in DMEM containing 10% (v/v) FBS and 1% pen-strep at 37 °C in a humidified atmosphere containing 5% $CO_2$. Analytically pure $Mg(NO_3)_2 \cdot 6H_2O$, $Al(NO_3)_3 \cdot 9H_2O$, NaOH, and ethanol were purchased from Beijing Chemical Co., Ltd. Deionized water was used in all the experimental processes.

**Preparation of ZnPc(1.5%)/LDH:** ZnPc(1.5%)/$Mg_2Al$-LDH photosensitizer was synthesized according to nucleation/crystallization separation method reported by our group.[1] Solution A: $Mg(NO_3)_2 \cdot 6H_2O$ (0.05 mol), $Al(NO_3)_3 \cdot 9H_2O$ (0.025 mol), ZnPc (0.337 g) dissolved in 100 mL of ethanol solution (2:3, v/v). Solution B: NaOH (0.15 mol) dissolved in 100 mL of deionized water. Solution A and B were added into colloid mill for 1 min with the speed of 3500 rpm. The obtained slurry was sealed in a Teflon-lined stainless steel autoclave and heated at 80 ºC for 24 h. The product was dried in vacuum at 60 ºC for 6 h after being washed by the distilled water and anhydrous ethanol for 3 times.

**Preparation of ZnPc-DOX/LDH:** the prepared ZnPc(1.5%)/LDH and 10 mL protonated DOX (0.0408 g/ml) was dissolved in 100 mL distilled water, the mixture was stirred for 24 h followed by washing with distilled water for 3 times and dried in vacuum at 60 ºC for 6 h.

**Release studies:** PBS solution (pH=7.4) was used to simulate intracellular conditions of normal cells. Acetic acid was additionally added to adjust the pH to 5.6 and 4.1

(physiological conditions of tumor). In a typical release test, ZnPc-DOX/LDH with a drug concentration of 5 μg/mL was dispersed throughly in appropriate medium (30 mL, pH=4.1) at 37 °C and stirred constantly. At selected time points, 2 mL of the suspension was collected and centrifuged at 10000 rpm. The supernatant and solid sample were measured *via* UV-vis absorption at 500 and 535 nm to determine the concentration of DOX (636 and 683 nm to determine the concentration of ZnPc), respectively.

**Detection of singlet oxygen:** Disodium salt of 9,10-anthracenedipropionic acid (ADPA) was used to detect singlet oxygen generated by ZnPc and ZnPc-DOX/LDH. Normally, an ADPA solution (150 μL, 7.5 mM) was added into the ZnPc and ZnPc-DOX/LDH suspension (3 mL, 10 μg/mL) and mixed thoroughly, followed by irradiation at 650 nm using a simulated sunlight source (optical filter 650±5 nm). The UV absorption curve was recorded every minute and the decrease rate of the UV absorption intensity at 378 nm is proportional to the produced amount of singlet oxygen.

***In vitro* studies on cancer cells:** KB cells were cultured and expanded in 25 $cm^2$ cell-culture flask. After reaching 80–90% confluence, the KB cells were washed with PBS, afterwards detached from the flask by addition of 1.0 mL of 0.25% trypsin for 1–3 min at 37 °C. KB cells ($1\times10^4$ cells/well) were seeded into two 96-well plates, respectively. To study the chemotherapy-PDT performance, cells were treated with different concentrations of ZnPc/LDH, DOX/LDH and ZnPc-DOX/LDH. After a further incubation of 24 h, cells were washed with PBS for 3 times. The plates were irradiated with a simulated sunlight source (optical filter: 650±5 nm; power density: 10 $mW/cm^2$) for 20 min. The colorimetric MTT was used to determine the cell viability. To study the photo- and dark-toxicity effect, KB cells ($1\times10^4$ cells/well) were seeded into two 96-well plates and then treated with DOX (5 μg/mL), ZnPc (5 μg/mL), ZnPc/LDH (equivalent ZnPc: 5 μg/mL), DOX/LDH (equivalent DOX: 5 μg/mL), ZnPc-DOX/LDH (equivalent DOX: 2.5 μg/mL; ZnPc: 2.5 μg/mL), respectively. After a further incubation of 24 h, cells were washed with PBS for 3 times. One plate was irradiated with a simulated sunlight source (optical filter: 650±5 nm; power density: 10 $mW/cm^2$) for 20

min and another was kept in the dark outside the incubator. The colorimetric MTT was used to determine the cell viability. In a typical cellular image experiment, the cells ($1\times10^4$ cells/well) were seeded into two 96-well plates respecitvely and incubated for 24 h. The cells were treated with 5 μg/ml of ZnPc, DOX, ZnPc/LDH, DOX/LDH, and ZnPc-DOX/LDH, and then incubated at 37 °C for another 24 h. After washing with PBS and irradiation for 20 min, treated cells were stained with propidium iodide (PI) and mounted for optical microscope examinations. Cells which were treated with the same procedure but without irradiation were also studied as a reference sample.

**Sample Characterization:** Powder X-ray diffraction patterns of the samples were collected on a Shimadzu XRD-6000 diffractometer using a Cu Kα source, with a scan step of 0.02° and a scan range between 3°–70°. The morphology of the samples was investigated using a scanning electron microscope (SEM; Zeiss SUPRA 55) with an accelerating voltage of 20 kV. The particle size distribution and zeta potential was determined using a Malvern Mastersizer 2000 laser particle size analyzer. The Fourier transform infrared (FT-IR) spectra were recorded using a Vector 22 (Bruker) spectrophotometer using the KBr pellet technique in the range 4000–400 $cm^{-1}$ with 1 $cm^{-1}$ resolution. The UV-vis absorption spectra were collected in the range 250–800 nm on a Shimadzu U-3000 spectrophotometer, with a slit width of 1.0 nm. The fluorescence spectra were performed on a RF-5301PC fluorospectrophotometer with the excitation wavelength of 630 nm. The fluorescence emission spectra range in 640−750 nm, and the width of both the excitation and emission slit is 3 nm. Fluorescence images of these samples were obtained using an Olympus1X71 fluorescence microscope with 400 folds enlargement.

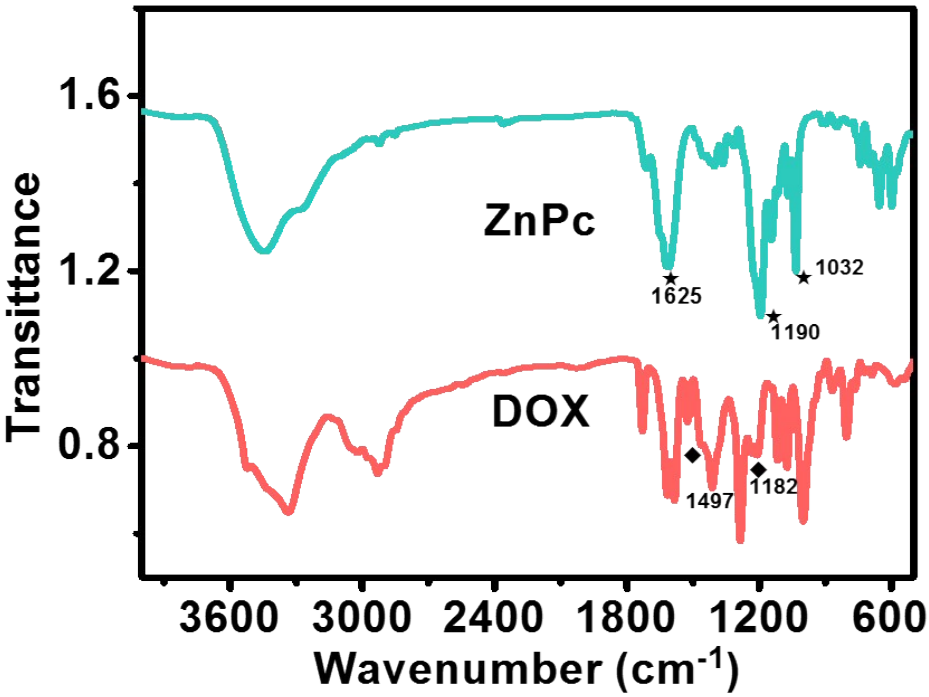



**Fig. S1** The FT-IR spectra of ZnPc and DOX.

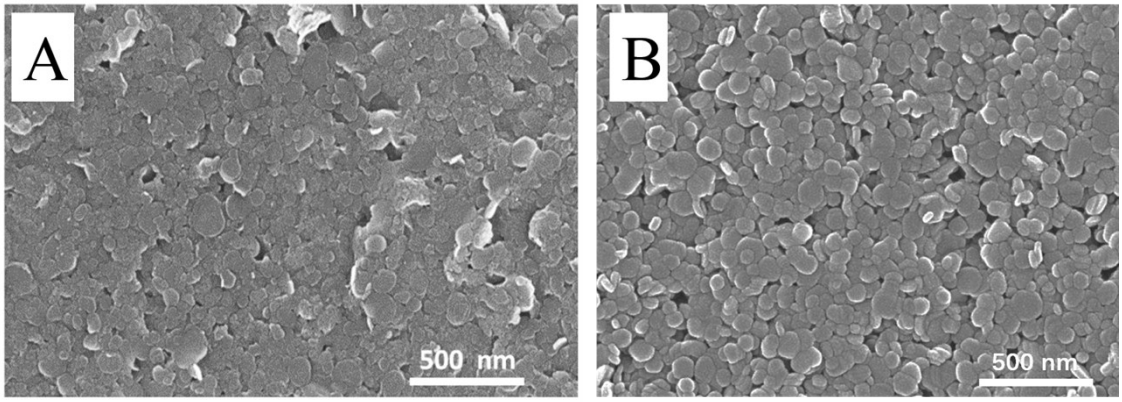



**Fig. S2** SEM images of (A) ZnPc/LDH and (B) DOX/LDH.

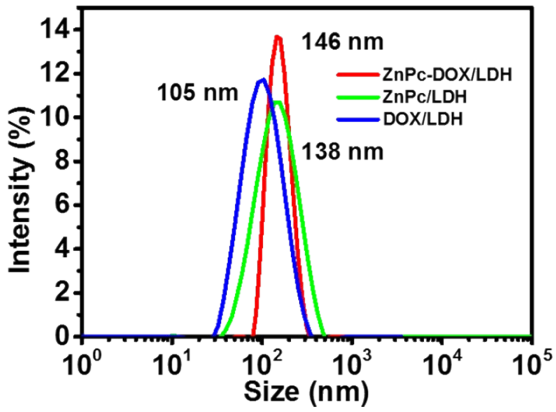
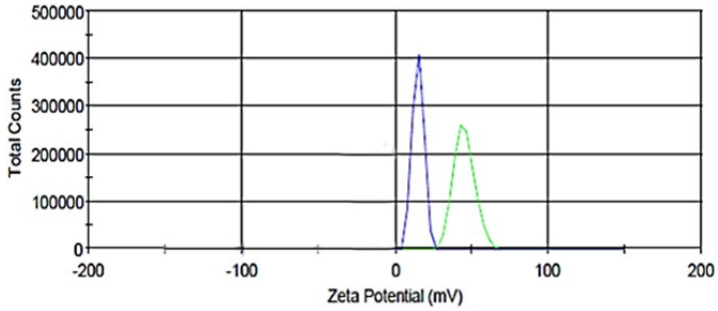



**Fig. S3** Particle size distribution of ZnPc/LDH, DOX/LDH and ZnPc-DOX /LDH.

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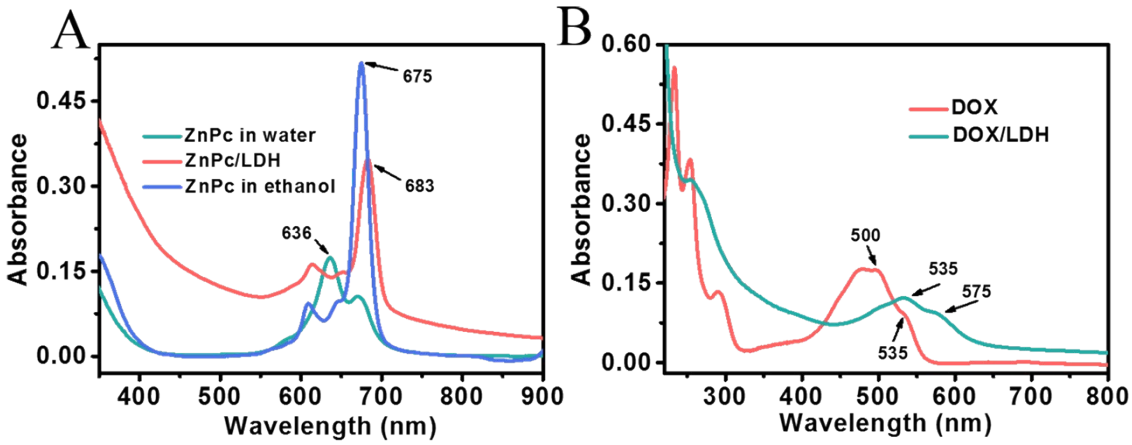


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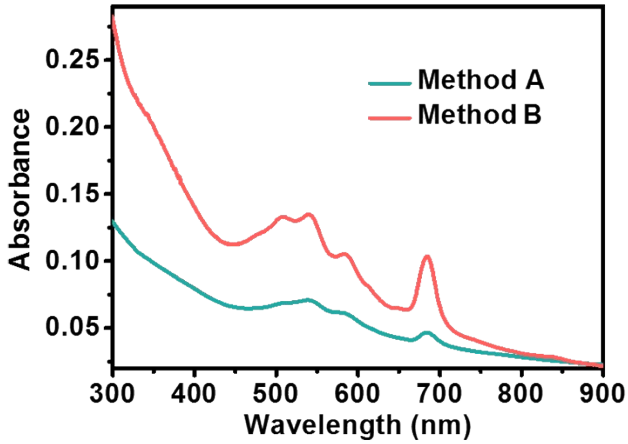


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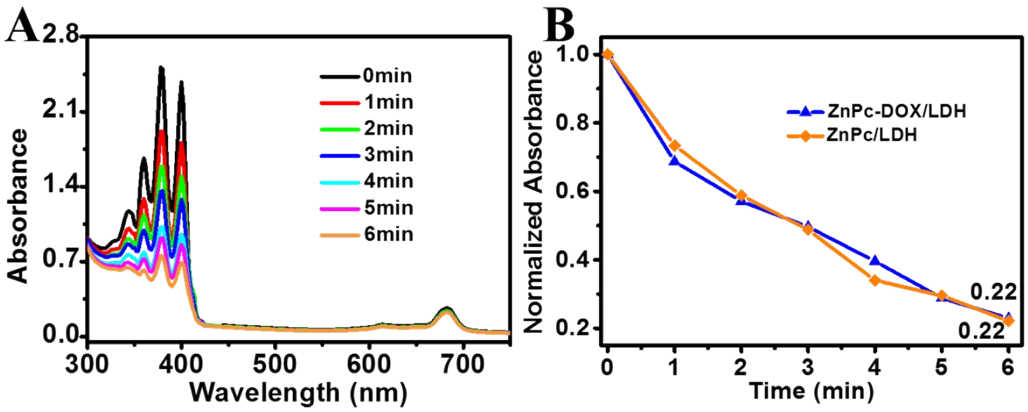


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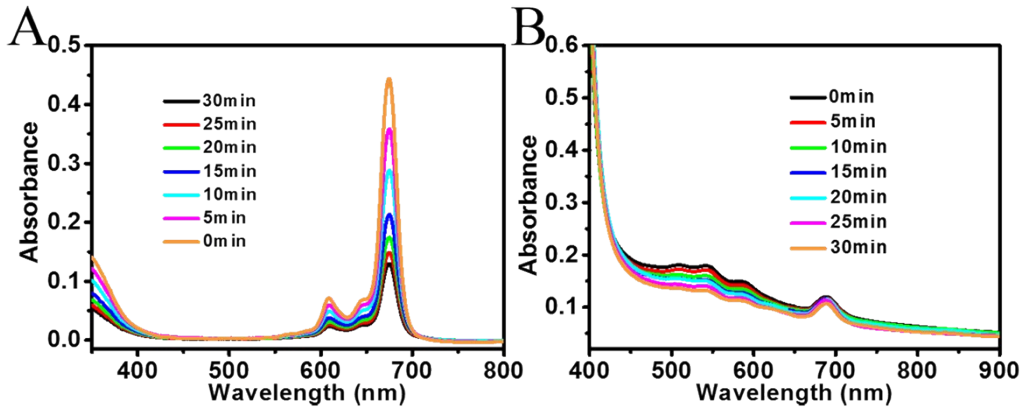


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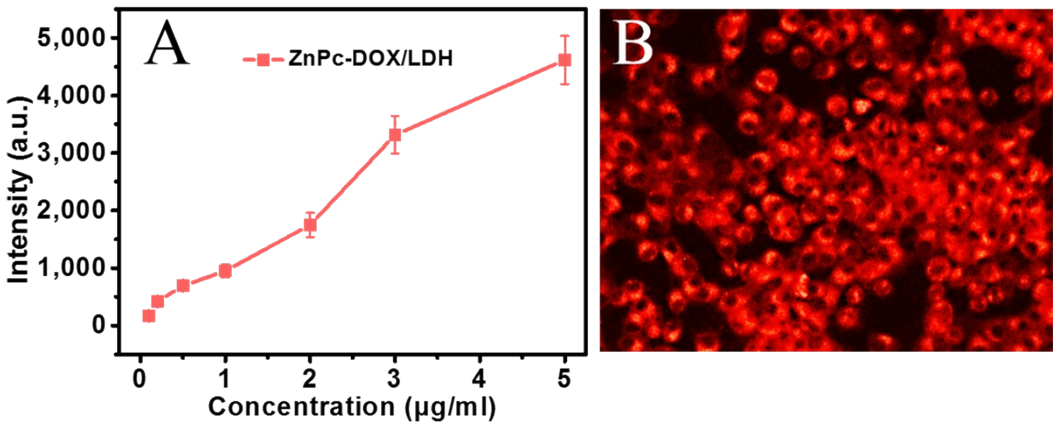


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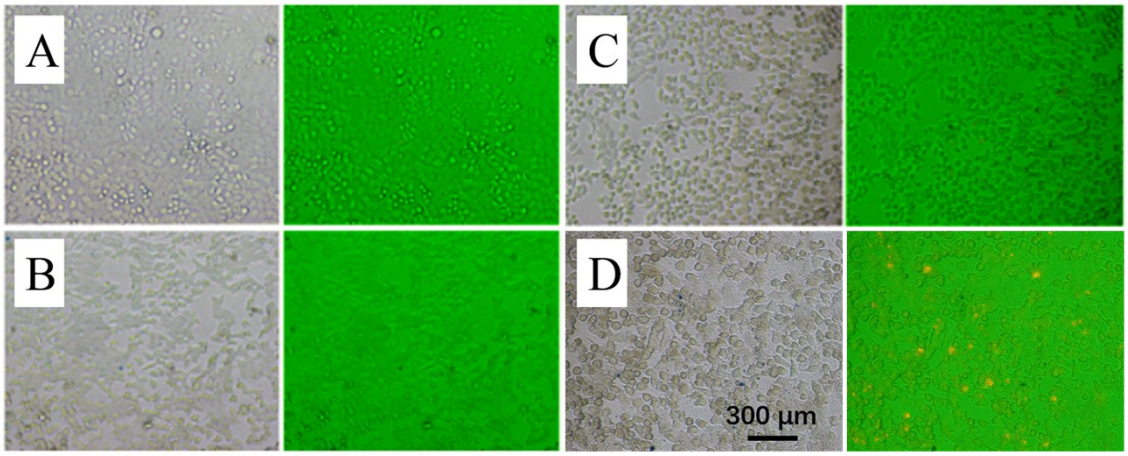


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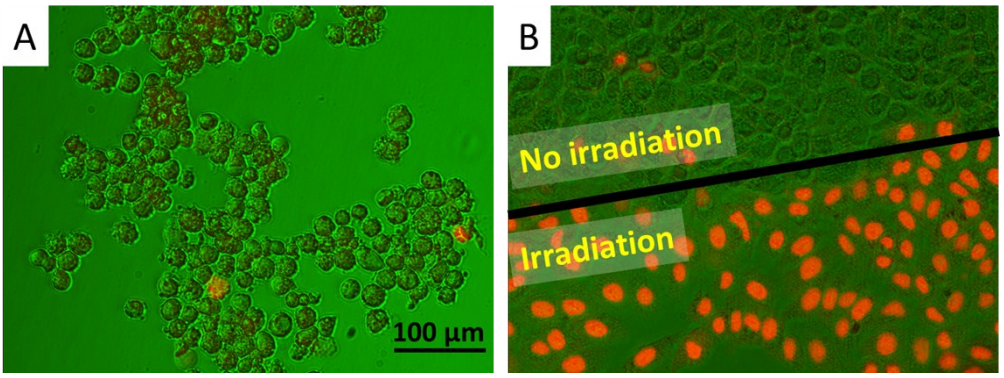


**Fig. S11** (A) Merged images of KB cells treated with ZnPc-DOX/LDH without irradiation (5 μg/mL, 24 h incubation); (B) Merged images of KB cells treated with ZnPc-DOX/LDH with partial irradiation (5 μg/mL, 24 h incubation).

**Reference:**

1. Y. Zhao, P. Li, R. Zhang, D. G. Evans and X. Duan, *Chem. Mater.*, 2002, **14**, 4286-4291.