Supporting Information for

A Monomeric Photosensitizer for Targeted Cancer Therapy

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Experimental section:

Materials: ZnPc, polyvinylpyrrolidone (PVP Mw=16000) and folic acid were purchased from sigma. Dulbecco's modified Eagles medium (DMEM) containing high glucose, L-glutamine, 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) was purchased from sigma. Human liver carcinoma (HepG2) 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.

Preparation of ZnPc(*x*%)/PVP and ZnPc(5.88%)-FA/PVP: series of ZnPc(*x*%)/PVP composite materials were synthesized *via* a facile liquid synthesis method. In brief, ZnPc and PVP with various mass ratios (ZnPc:PVP = 1:1, 1:2, 1:4; 1:8; 1:16, respectively) were simultaneously added into a centrifuge tube, followed by shaking for 12 h. The ZnPc(5.88%)-FA/PVP was prepared by a modified method based on previous report.[1] Firstly, ZnPc(5.88%)/PVP solution was prepared as described above. Subsequently, the protonated FA was slowly added into the ZnPc(5.88%)/PVP colloidal solution. Since the protonated FA is water-soluble while neutral FA is hydrophobic, the solution pH was adjusted with dilute hydrochloric acid very carefully and the protonated FA returned to hydrophobic FA, which can be encapsulated into ZnPc(5.88%)/PVP micelle *via* a strong ultrasonic treatment and vigorous

stirring for 12 h. This is a key step to prepare the stable ZnPc-FA/PVP colloidal solution: the dilute hydrochloric acid was added dropwise and the pH value was precisely adjusted to be neutral (pH=7.0). Finally, the ZnPc(5.88%)-FA/PVP micelle was purified by dialysis against pure water for 24 h (molecular weight cut off 3500) to remove the unbound ZnPc and FA.

Detection of Singlet Oxygen: the generation of singlet oxygen for the ZnPc(*x*%)/LDH photosensitizers was detected chemically using disodium salt of 9,10-anthracenedipropionic acid (APDA) as a singlet oxygen sensor.[2] An APDA solution (150 μL, 7.5 mM) was added into the ZnPc(*x*%)/PVP suspension (10 μg/mL, 3 mL) and mixed thoroughly, followed by irradiation at 650 nm using a simulated sunlight source with optical filter (650±5 nm). The decrease rate of the UV absorbance at 378 nm is proportional to the amount of singlet oxygen produced, and the absorbance value was recorded per 2 min.

***In vitro* Studies on Tumor Cells:** HepG2 cells were grown and expanded in 25 cm^2 cell-culture flask. After reaching 80–90% confluence, the HepG2 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. HepG2 cells (1×10^4 cells/well) were seeded into two 96-well plates, respectively. To study the optimal FA capacity, the cells were then treated with different concentrations of ZnPc(5.88%)-FA/PVP suspension (ZnPc: 10 μg/mL; FA: 10–160 μg/mL). After a further incubation of 24 h, the plate was washed by PBS for 3 times and the fluorescence spectra of these cell lysate samples were recorded. To investigate the optimal irradiation time, the cells were treated with 10 μg/mL of ZnPc(5.88%)-FA/PVP suspension. After a further incubation of 24 h, the plate was irradiated with a simulated sunlight source (optical filter 650±5 nm) for different time. The colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

(MTT) was used to determine the cell viability. The study on incubation time was performed by the same method.

To study the PDT performance, cells were treated with different concentrations of ZnPc(*x*%)-FA/PVP colloid. 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 30 min. The colorimetric MTT was used to determine the cell viability. The PDT performance of ZnPc/PVP and ZnPc were performed by the same method. For the study of the phototoxicity effect, HepG2 cells (1×10^4 cells/well) were seeded into two 96-well plates and then treated respectively with ZnPc(5.88%)-FA/PVP (equivalent ZnPc: 10 μg/mL; FA: 40 μg/mL; PVP: 160 μg/mL), ZnPc (10 μg/mL), FA (200 mg/mL), PVP (200 mg/mL) suspension/solution. 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 30 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 (1×10^4 cells/well) were seeded into two 96-well plates respectively and incubated for 24 h. The cells were treated with 10 μg/mL of ZnPc(5.88%)/PVP, ZnPc(5.88%)-FA/PVP, pristine ZnPc and blank, followed by an incubation at 37 °C for another 24 h. After washing with PBS and irradiation for 0.5 h, treated cells were stained with propidium iodide (PI) and mounted for optical microscope examinations. Cells treated with the same procedure but without irradiation were also studied as a reference sample.

Animal Experiments: Male Balb/c mice (Balb/c-nu, ~25 g) were purchased from Academy of Military Medical Science and used under protocols approved by 302th Military Hospital Animal Research Center. 2×10^6 HepG2 cells suspended in 200 μL phosphate buffered saline (PBS) were subcutaneously injected into the hind flank of each male Balb/c mouse. The mice bearing HepG2 tumors were treated when the tumor volume reached ~40 mm^3.

***In vivo* Photodynamics Therapy:** Mice were randomized into four groups of 8 animals per group for the following treatments: (Group i) 200 μL of saline injected intravenously with irradiation at 36 J/cm^2; (Group ii) 200 μL of ZnPc(5.88%)/PVP (equivalent 50 μg ZnPc) injected intravenously with irradiation at 36 J/cm^2; (Group iii) 200 μL of ZnPc(5.88%)-FA/PVP (equivalent 50 μg ZnPc) injected intravenously with irradiation at 36 J/cm^2; (Group iv) 200 μL of ZnPc injected intravenously with irradiation at 36 J/cm^2. Mice with the PDT were irradiated with a simulated sunlight source (optical filter: 650±5 nm; power density: 20 mW/cm^2) for 30 min (i.e., an optical fluence rate of 36 J/cm^2). The tumor size was measured by a caliper every day and calculated as the volume = (tumor length)×(tumor width)^2×0.5.[3] Relative tumor volume was calculated as V/V_0 (V, V_0 are the tumor volume measured at time t and t_0, respectively).

Near-IR Fluorescence Images and Histology Examination: For *in vivo* near-IR fluorescence imaging, the mice were received intravenous injection of ZnPc(5.88%)/PVP and ZnPc(5.88%)-FA/PVP solution with a dose of 50 μg. Near-IR fluorescence images were obtained at 0, 1, 2, 3, 4, 6, 8 and 24 h after injection. For histological examination, tumors from the treated group and control groups were fixed in 4% formalin and conducted with paraffin embedded sections for H&E staining. The slices were examined by a digital microscope (Olympus).

Sample Characterization: The solid UV-vis absorption spectra were collected in the range 200–900 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 675 nm. The fluorescence emission spectra range in 680–750 nm, and the width of both the excitation and emission slit is 3 nm. The morphology of the samples was investigated by the Philips Tecnai 20 Transmission electron microscopy (TEM) with the accelerating voltage 200 kV. The particle size distribution was determined using a Malvern Mastersizer 2000 laser particle size analyzer. Fluorescence images of these samples were obtained using an Olympus 1X71 fluorescence microscope with 400 folds enlargement. Near-IR fluorescence images were obtained using an IVIS Lumina fluorescence imaging system (Cy5.5 channel, λ_{ex}= 615–665 nm, λ_{em}= 695–770 nm).

Figure S1. UV-vis absorption spectra of ZnPc loaded on different polymers (ZnPc concentration: 10 μg/mL; loading amount: 5.88%).

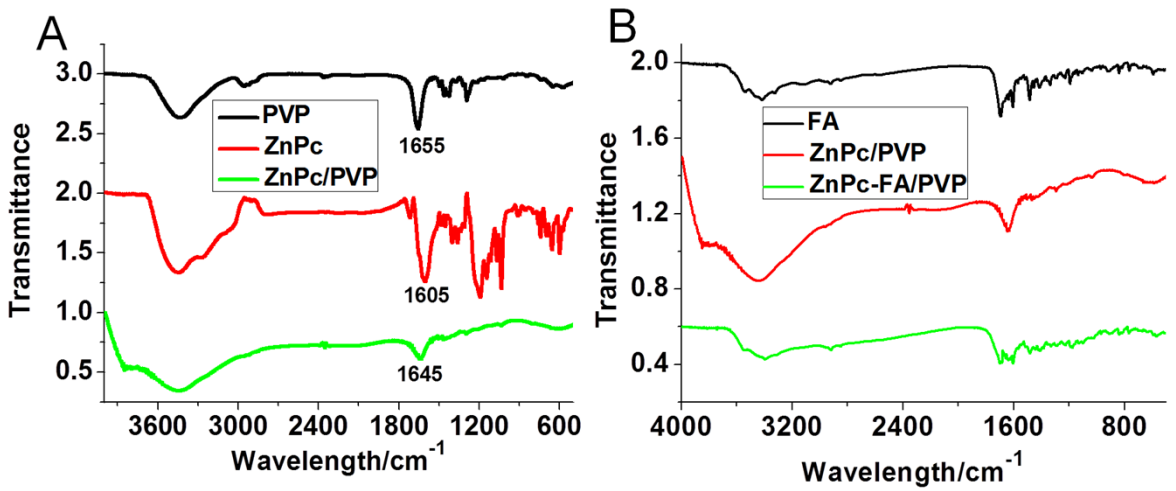


Figure S2. (A) FT-IR spectra of PVP, ZnPc and ZnPc/PVP; (B) FT-IR spectra of FA, ZnPc/PVP and ZnPc-FA/PVP.

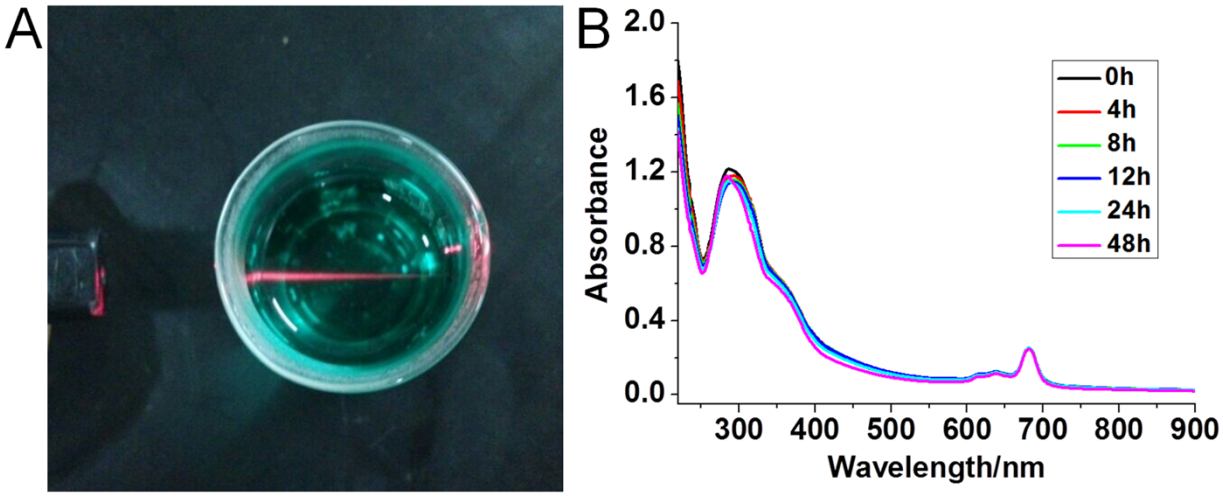


Figure S3. (A) The Tyndall effect of ZnPc(5.88%)-FA/PVP, indicating the formation of colloid. (B) UV-vis absorption spectra of ZnPc(5.88%)-FA/PVP colloidal solution in the dialysis test for 48 h (molecular weight cut off 3500).

In order to verify the formation of ZnPc-FA/PVP colloidal system and its stability, the purified ZnPc-FA/PVP micelle solution was loaded into a dialysis bag (molecular weight cut off 3500) and dialyzed against pure water. UV-vis spectroscopy was used to monitor the micelle solution. No obvious change in the absorption band of folic acid (289 nm) and ZnPc (682 nm) is observed during the 48 h dialysis test (Figure S3), indicating both folic acid and ZnPc are encapsulated in PVP micelle stably.

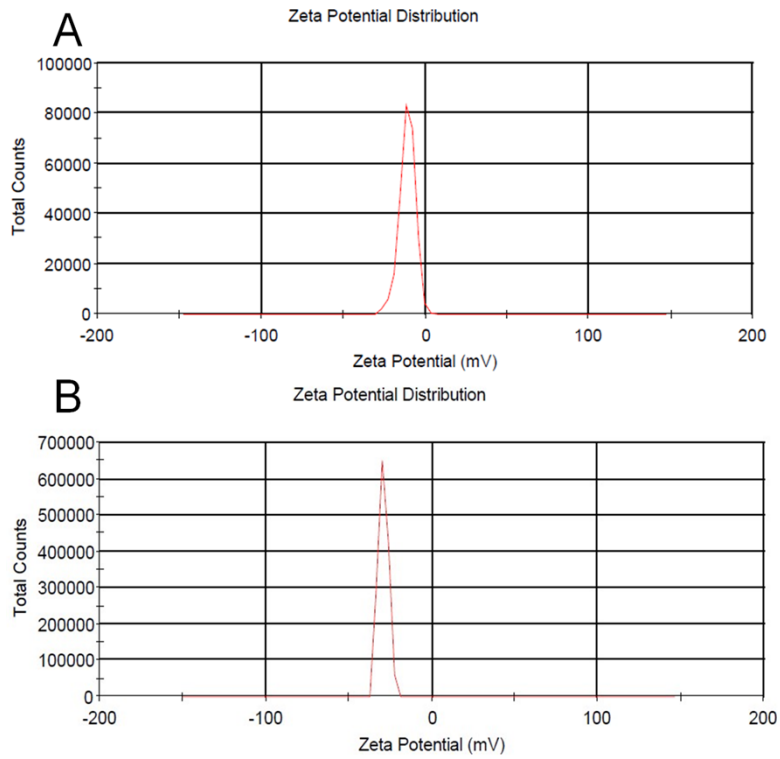


Figure S4. The Zeta potential for the samples of ZnPc(5.88%)/PVP (A) and ZnPc(5.88%)-FA/PVP (B).

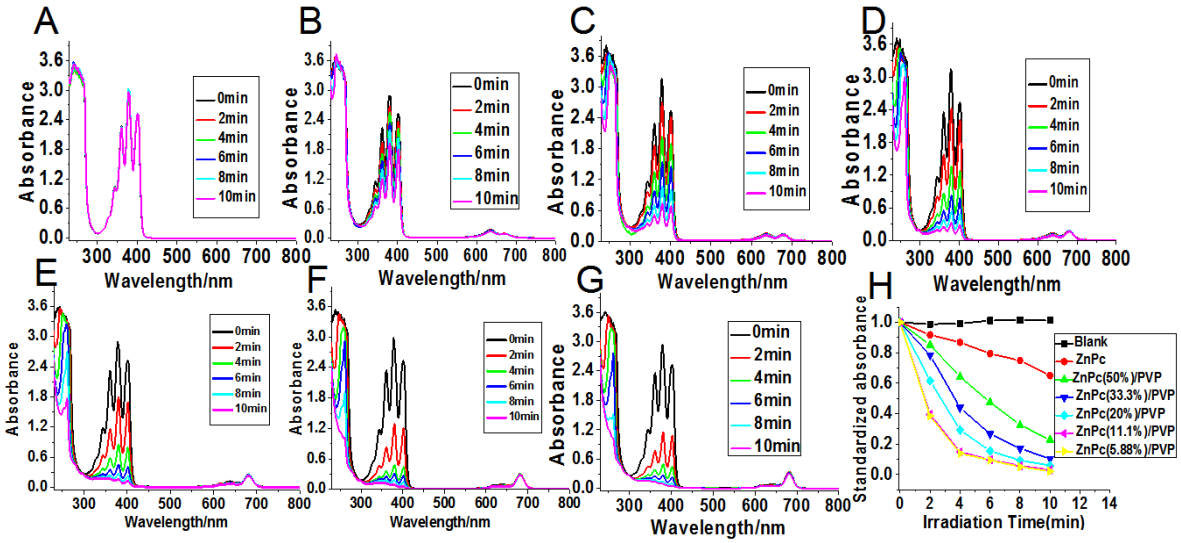
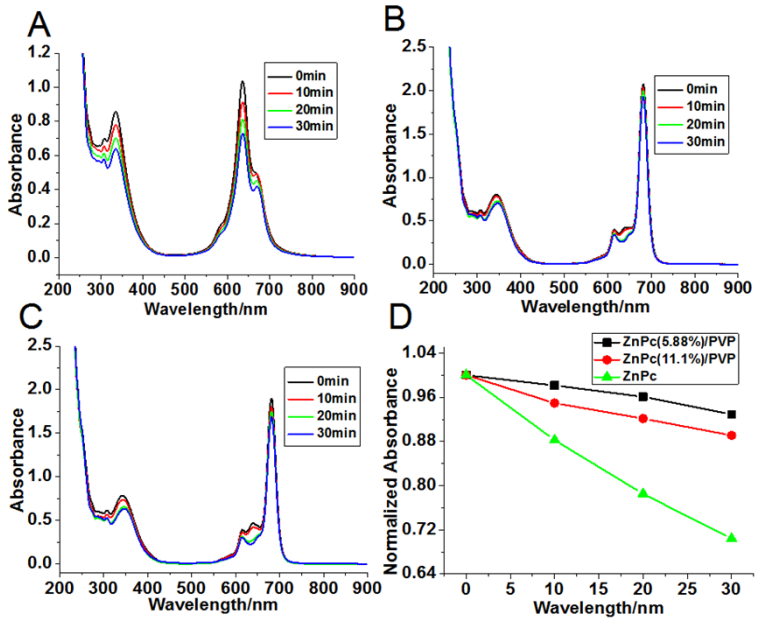


Figure S5. Decay curves of ADPA absorbance in the presence of (A) blank, (B) ZnPc, (C) ZnPc(50%)/LDH, (D) ZnPc(33.3%)/LDH, (E) ZnPc(20%)/LDH, (F) ZnPc(11.1%)/LDH, (G) ZnPc(11.1%)/LDH and (H) the normalized decay curves for ADPA absorbance at 378 nm in the presence of pristine ZnPc, ZnPc(*x*%)/PVP and blank measurement as a function of irradiation time (650 nm).



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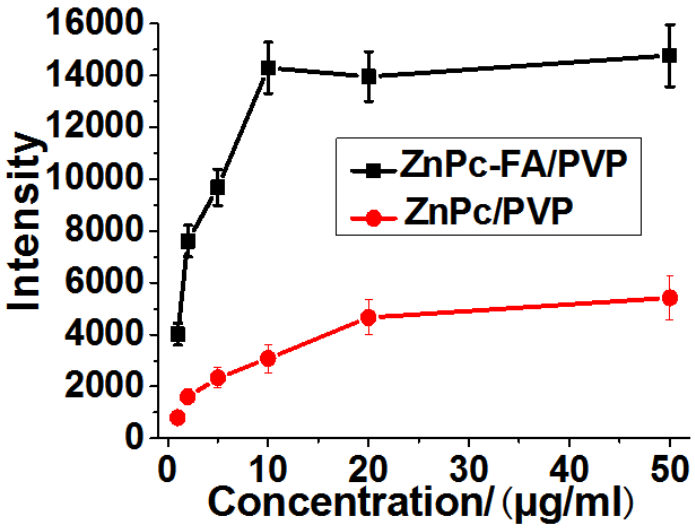


Figure S9. The fluorescence intensity of cell lysate samples from HepG2 cells incubated with various concentrations of ZnPc(5.88%)-FA/PVP and ZnPc(5.88%)/PVP.

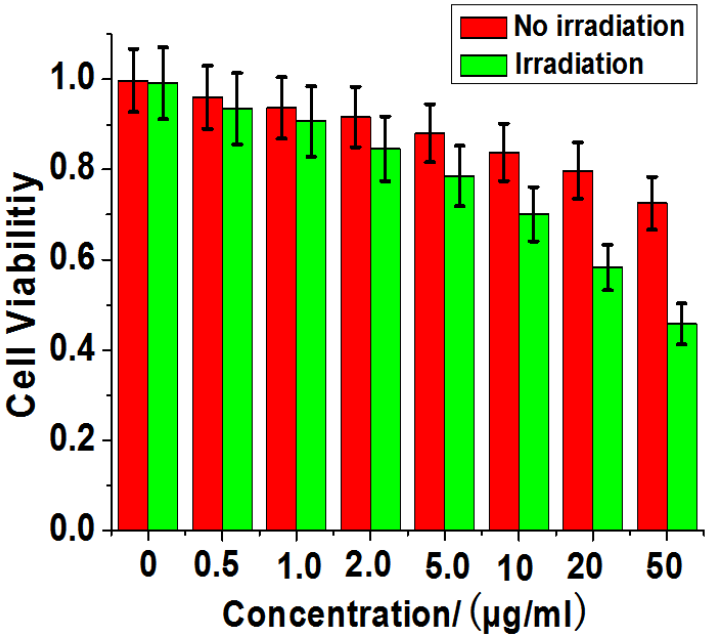
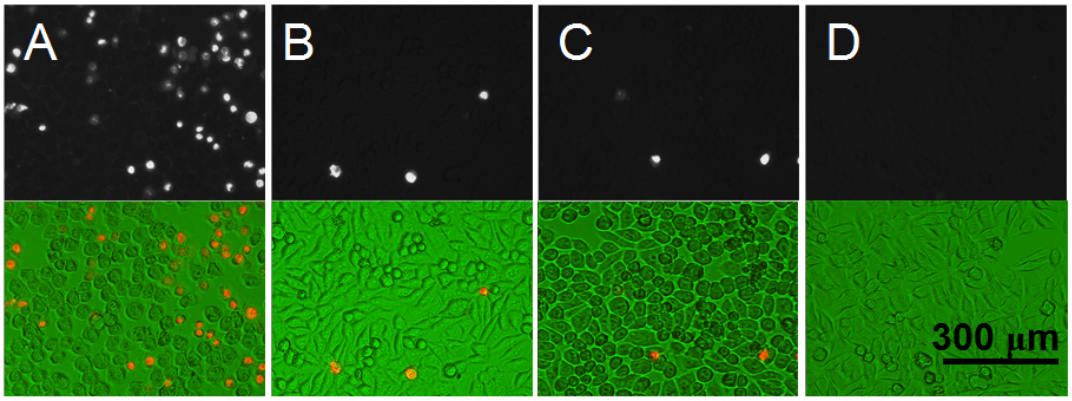


Figure S10. The PDT performance of ZnPc with various concentrations.



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