# Supporting Information

# Doxorubicin loaded chitosan-ZnO hybrid nanospheres combining cell imaging and cancer therapy

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**Experimental Section**

## Materials

Water-soluble chitosan (WCS) with a number average molecular weight ($M_n$) of 5,000 Da was purchased from Yuhuan Biomedical Company (Zhejiang, China) and used without further purification. Ethanol (99.5%), zinc acetate dehydrate ($Zn(OAc)_2 \bullet 2H_2O$), diethanolamine (DEA), oleic acid (OA), Ethylenediamine tetraacetic acid (EDTA) and glutaraldehyde (GA) were purchased from Aldrich. Doxorubicin hydrochloride (DOX) was obtained from Tecoland Corporation (USA). All other ingredients were of analytical grade unless otherwise stated.

## Characterization

Photoluminescence (PL) spectra were taken with Horiba Fluorolog-3 Spectrofluorometer and surface plasmon resonance properties were explored by UV-Vis-NIR spectrophotometer (Shimadzu UV-3600). Morphology of the as-synthesized NPs was investigated by transmission electron microscopy (TEM, JEOL TEM-100) and high resolution TEM (HR-TEM) (JEOL, TEM-2100). The X-ray diffraction (XRD) pattern was recorded in the 2ɵ ranging from 20 to 60° on a Hitachi X-ray

diffractometer using Cu k1 radiation (λ=1.54056 Å) at 40 kV and 200 mA. Dynamic light scattering (DLS) using a Zetaplus (Brookhaven Instruments Corporation, USA) was applied to observe the size distribution and zeta potentials of the NPs. The scattered light of a vertically polarized He-Ne laser was collected by auto correlator at an angle of 90°. For zeta potential analysis, each sample was adjusted to a concentration of 0.1% (w/v) in deionized water and sonicated before measurement. All measurements were carried triplicate and the values were the mean value. The element composition and nanostructure of the nanoparticles were further confirmed by the Field-Emission Scanning Electron Microscope (FE-SEM, JEOL JSM-6700F, Japan) equipped with Energy Dispersive Spectrometer(EDS).

**Synthesis of ZnO QDs**

To prepare water-dispersed ZnO QDs, 37.2 mg zinc nitrate hexahydrate, 26.3 mg DEA and 0.35 mmol OA were dissolved in 50 mL distilled water following the procedures according to the literature.[8] The mixture was allowed to react for 30 min at room temperature under vigorous stirring in a covered flask, and then the temperature increased slowly to 80 $^{o}$C with continuous stirring for another 3 hours. During this procedure, the mixture turned from colorless to milk white, indicated the formation of the ZnO nanocrystals. Finally, the obtained dispersion was cooled down to room temperature and the stable ZnO QDs were filtered (paper filter, 5 μm), centrifuged (25,000 rpm) and re-dispersed for further use.

**Synthesis of CS-ZnO Hybrid Nanospheres (CZNPs)**

Briefly, 12 mg of EDTA was added into 10 mL aqueous solution containing 25 mg CS and then the solution was stirred until the mixture was completely dissolved. After that, a predetermined amount of ZnO QDs was subsequently added to the CS-EDTA solution and the resultant solution was stirred for several minutes at the room temperature. As a non-solvent for both CS and EDTA, ethanol was added dropwise to the solution under constantly stirring until the clear solution turned to cloudy, which implied that ZnO QDs were successfully encapsulated into the CS-EDTA colloidal particle. After that, 100 µL of GA aqueous solution (25%) was added into the solution in order to cross-link the obtained hybrid nanospheres for 4 hours. The suspension was filtered (paper filter 5µm) and then dialyzed against deionized water for 24 hours to remove unreacted materials especially EDTA component. Finally, the CZNPs were harvested by centrifugation (10,000 rpm) for further use.

**Synthesis of DOX- loaded CS-ZnO Hybrid Nanospheres**

To get the DOX-loaded CZNPs, 5 mg of CZNPs were dispersed in 5 mL of DOX aqueous solution (1mg/mL). The reaction was carried out under continuous stirring at room temperature and protected from being lightened. After 24 hours incubation, the DOX was absorbed on the surface of CZNPs due to electrostatic forces between amine groups of chitosan and carboxyl groups of DOX. The DOX-loaded CZNPs were separated from the aqueous solution by centrifuge. Loading efficiency was analyzed by determining the amount of free drugs in supernatant with UV-Vis spectroscopy.

Intensity of characteristic absorption of DOX at 480 nm was measured, and the amount of DOX was quantitatively determined via standard curve. The DOX encapsulation efficiency was calculated as follows:

$$\text{encapsulation efficiency}\,(\%) = \frac{\text{weight of the drug in nanospheres}}{\text{weight of the initial quantity}} \times 100\%$$

**The quantum yield of ZnO QDs and CZNPs**

The quantum yields of ZnO QDs and CZNPs were measured according to the literature using quinine sulfate as a references (1). Briefly, Quinine sulfate was dissolved in 0.5 H2SO4 to prepare the standard solution with five concentrations. The photoluminescence intensities (excited at 350 nm) of these quinine sulfate solution were measured with Horiba Fluorolog-3 Spectrofluorometer. After that, ZnO QDs solutions with five different concentration were also prepared and their photoluminescence intensities were obtained. The quantum yield was calculated using the below equation:

$$\phi_{ZnO}=\phi_{ST}(0.55)(m_{ZnO}/m_{ST})(\eta^2_{ZnO}/\eta^2_{ST})$$

where $\phi_{ZnO}$ is the quantum yield, and m is slope. Detailed explanation can be found in the literature (1).

**Cytotoxicity Assay**

Cytotoxicity of CZNPs and DOX-loaded CZNPs was evaluated via a standard MTT assay, using human alveolar basal cell line A549. The cells were seeded into 96-well plates at a density of $10^4$ cells per well and incubated at 37 $^oC$ in 5% $CO_2$ atmosphere. The medium was modified Dulbecco's medium and changed every other day until 80% confluence reached. The medium of each well was replaced with 100 μL fresh

medium containing CZNPs or DOX-loaded CZNPs solution with different concentrations for 24 hours and 48 hours incubation. After that, the cells were washed twice with phosphate-buffered saline (PBS, PH 7.4) and 20 µL of 2.5 mg/mL of MTT solution was added and incubated for 4 hours. The viable cells were re-suspended in 200 µL of dimethyl sulfoxide (DMSO) and the absorbance of individual wells was measured at 490 nm by an iMark Enzyme mark instrument (BIO-RAD Inc. USA).

**Confocal Laser Scanning Microscopy (CLSM)**

A549 cells were incubated in confocal imaging specific petri-dish (Biotek) at 37 $^{o}$C with 5% $CO_2$ at a density of 3 x $10^5$ cells per well containing DMEM medium supplemented with 10% FBS and 1% penicillin-streptomycin. ZnO QDs，CZNPs and DOX-loaded CZNPs were then separately introduced into the system for 4 hours. The medium was then discarded, and the cells were washed with PBS suspension at least thrice to remove unbound NPs. After that, the lysosomes and nucleus of cells were stained by Lysotracker Red and SYTO. Finally, the cells were fixed by formaldehyde and observed using a laser scanning confocal microscope (LSCM, Carl Zeiss, LSM510 Meta). Acquired fluorescence images were further processed by Image Pro Plus software (Media Cybernetics). Excitation of the DOX, ZnO QDs were performed with lasers at 488 nm and 350 nm, and emission spectra were collected using a wavelength range of 570-630 nm and 450-500 nm respectively.

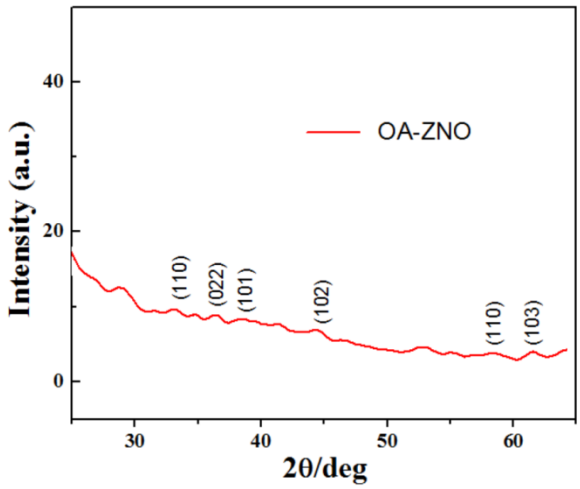


Fig. S1 XRD patterns of ZnO QDs

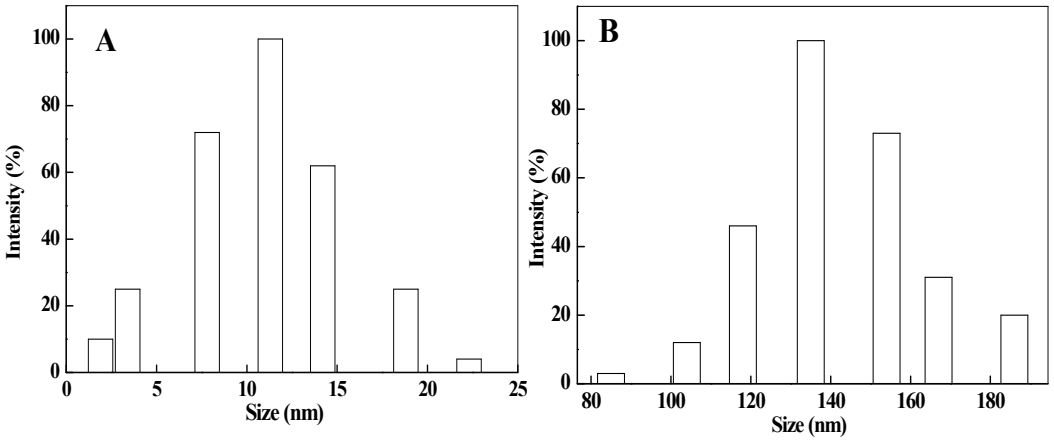


Fig. S2, The hydrodynamic diameter of ZnO QDs (A) and CZNPs (B)

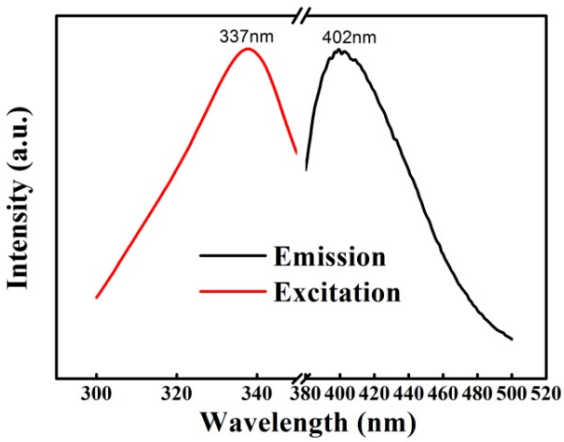


Fig. S3, PL and PLE spectra of ZnO QDs solution. Samples were excited by 350 nm light.

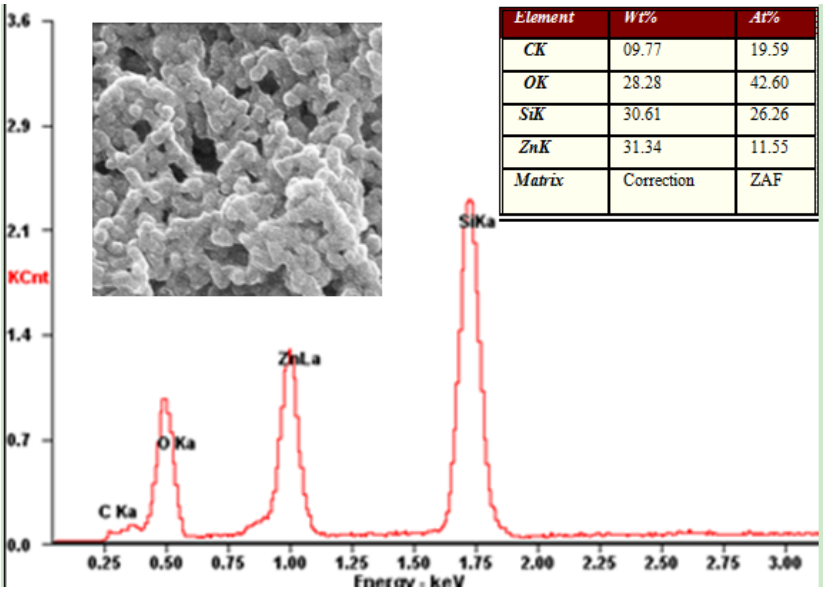


Fig. S4, Elemental analysis of CZNPs using SEM equipped with EDS

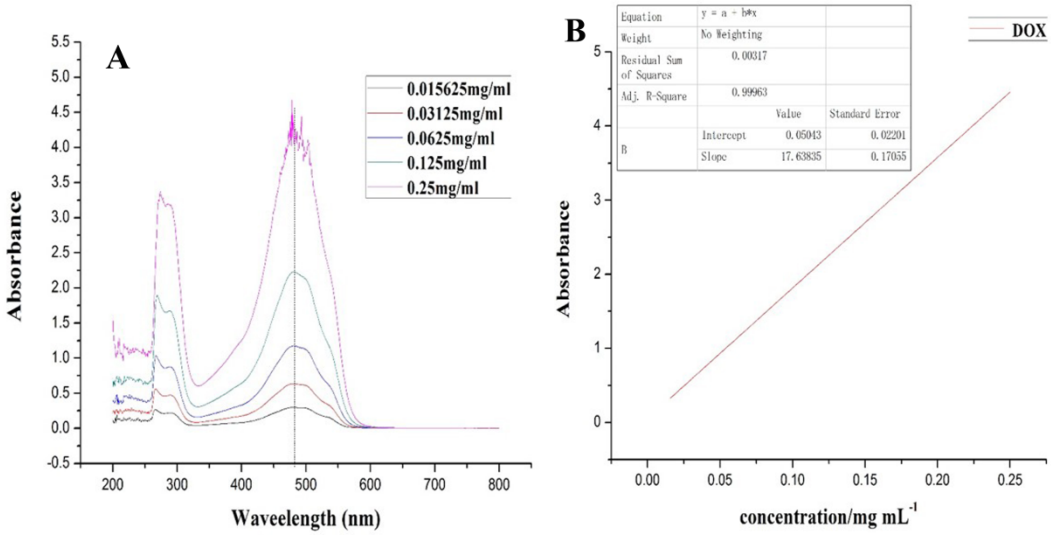


Fig. S5, the UV absorption curves of standard DOX solution with different concentration (left), and the calibration line (right)

(1) Ying-Song Fu, Xi-Wen Du, Sergei A. Kulinich, Jian-Sheng Qiu, Wen-Jing Qin, Rui Li, Jing Sun, and Jim Liu, Stable Aqueous Dispersion of ZnO Quantum Dots with Strong Blue Emission via Simple Solution Route, JACS, 2007,129, 16029-16033