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Supporting Information for

An ultrathin photosensitizer for simultaneous fluorescence imaging and photodynamic therapy

Tongyang Hu,[a] Jun He,[b] Shaomin Zhang,[a] Xuan Mei,[a] Weiku Zhang,*[b] Ruizheng Liang,*[a] Min Wei,*[a] David G. Evans,[a] and Xue Duan[a]

[a] T. Hu, S. Zhang, X. Mei, Dr. R. Liang, Prof. M. Wei, Prof. D. G. Evans, Prof. X. Duan
State Key Laboratory of Chemical Resource Engineering
Beijing Advanced Innovation Center for Soft Matter Science and Engineering
Beijing University of Chemical Technology
Box 98, Beijing 100029, P. R. China
Fax: (+86)10-6442-5385
E-mail: Liangrz@mail.buct.edu.cn (R. Liang); weimin@mail.buct.edu.cn (M. Wei)

[b] Dr. J. He, Dr. W. Zhang
Institute of Clinical Medical Sciences & Department of Pharmacy,
China-Japan Friendship Hospital,
Beijing 100029, P. R. China,
Tel:86-10-84205662;
E-mail: zhangweiku@zryhyy.com.cn (W. Zhang).

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Experimental Section

1. Preparation of materials

1.1 Materials. Magnesium nitrate ($Mg(NO_3)_2•6H_2O$), aluminium nitrate ($Al(NO_3)_3•9H_2O$), sodium hydroxide (NaOH), sodium nitrate ($NaNO_3$), formamide, and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) were purchased from Aladdin Chemical. Co. Ltd (shanghai, China). Calceinacetoxymethyl ester (Calcein-AM), propidium iodide (PI), hochest 33342 and 9, 10-Anthracenedipropionic acid (ADPA) were purchased from Sigma-Aldrich Co. Ltd (St. Louis, MO, USA). Chlorin e6 (Ce6) was obtained from J&K Scientific (China). High glucose Dulbecco's modified Eagles medium (DMEM) 0.25% and trypsin-EDTA were purchased from Gibco (Invitrogen, Carlsbad, CA). Fetal bovine serum (FBS) was purchased from Excell Bio. Co., Ltd. (Shanghai, China). Phosphate-buffered saline (PBS) was purchased from Solarbio Science & Technology Co, Ltd (Beijing, China). Hela cells were obtained from the Institute of Basic Medical Sciences Chinese Academy of Medical Sciences. All other reagents and solvents were of analytical purity and used without further purification.

1.2 Synthesis of LDH nanosheets. LDH nanosheets were prepared by a facile method reported by our group previously.[1] In brief, solution A: $Mg(NO_3)_2•6H_2O$ (0.0004 mol) and $Al(NO_3)_3•9H_2O$ (0.0002 mol) dissolved in deionized water (40 mL). Solution B: NaOH (0.0045 mol) dissolved in deionized water (30 mL). Solution C: $NaNO_3$ (0.0002 mol) dissolved in 25% volume fraction of formamide solution (40 mL). Solution A and solution B were dropped into solution C simultaneously with stirring at 80 ℃ for 30 min. The resulting precipitate (LDH nanosheets) was centrifuged and washed 3 times with deionized water, followed by a further

dialysis (3 kDa) to remove the residual formamide.

1.3 Synthesis of CDs. 0.1 g of p-hydroxybenzoic acid and 50 μL of ethylenediamine were dissolved in deionized water (10 mL), followed by an ultrasonic dispersion for 10 min to obtain a homogeneous solution. Then the mixture was transferred into Teflon-lined autoclave and heated at 180 ℃ for 6 h. The obtained light yellow suspension was cooled and dialyzed to obtain purified CDs.

1.4 Synthesis of CDs-Ce6/LDH. Ce6 was dissolved in PBS. Ce6 solution and CDs suspension with various molar ratios were added into a suspension of LDH, stirring at room temperature for 12 h in dark. The mixture was centrifuged at 10000 r/min for 3 times and washed thoroughly with deionized water. Finally, the precipitate was resuspended in deionized water and stored at 4 ℃ in dark. The content of Ce6 was determined by UV-vis absorption spectroscopy based on the absorption peak at 660 nm using a standard curve method.

1.5 Detection of ROS production. 9,10-Anthracenedipropionic acid (ADPA) was used to measure the capability of various formulations of ROS production upon irradiation. In brief, an ADPA solution (150 μL, 7.5 mM) was added into 3 mL of water, CDs, LDH nanosheets, Ce6 and CDs-Ce6/LDH suspension, respectively, followed by an irradiation through a simulated sunlight source (optical filter 650 nm, 15 mW/cm^2) for 6 min. UV-vis absorption spectra were recorded every minute and the decrease rate of optical density at 378 nm was proportional to the ROS production efficiency.

1.6 Cell Line and Culture. Hela cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37 ℃ under 5% CO_2. The biocompatibility of CDs and LDH by MTT assay was studied before

in vitro fluorescence imaging and photodynamic therapy.

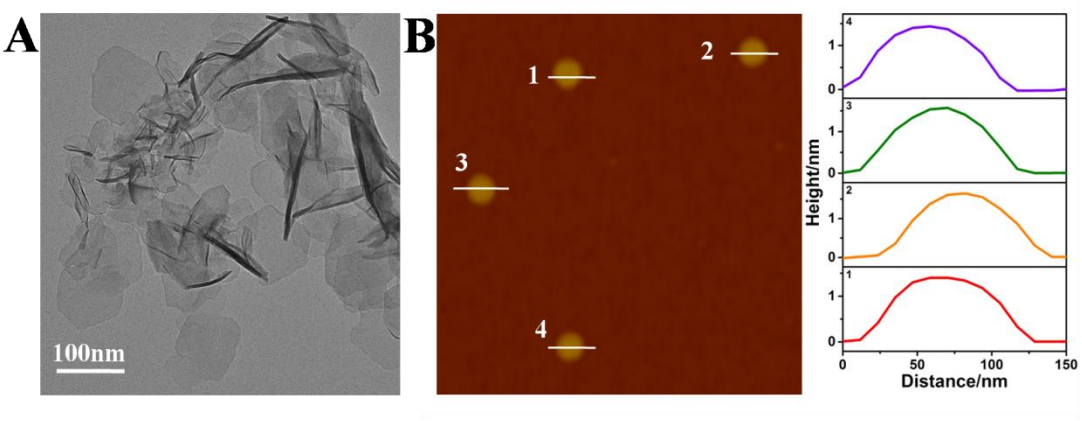
1.7 *In vitro* Fluorescence Imaging. Hela cells were cultured into 35 mm confocal dishes and incubated for 24 h. After incubating with pristine CDs, Ce6, CD-Ce6/LDH for 6 h, the cells were washed with PBS for 3 times thoroughly. The nuclei of cells were stained with hochest 33342 for 10 min and washed with PBS again for 3 times. A Leica TCS SP8 confocal laser scanning platform was used to capture the fluorescent images.

1.8 *In vitro* Photodynamics Therapy. Hela cells (1×10^4 well^{-1}) were seeded in two 96-well plates and cultured for 24 h. Then, cells were treated with 100 μL of medium containing serious of pristine CDs (0–10 μg/mL), Ce6 or CDs-Ce6/LDH (equivalent Ce6 0–10 μg/mL). After 24 h incubation, the cells were rinsed again with PBS thoroughly. 200 μL of fresh culture medium was added to each well and cell were treated with or without 650 nm irradiation (15 mW/cm^2, 20 min). After another 6 h incubation, the viability of cells was detected by the MTT assay. 20 μL of MTT solution (5.0 mg/mL) was added to each well for incubating another 4 h at 37 ℃. Subsequently, the medium was removed and 150 μL of dimethylsulfoxide (DMSO) was added to dissolve the purple formazan crystals formed by the living cells. Then the plates were gently shaken for 10 min in dark before measuring the absorption at 490 nm. The cell viability was determined by the ratio of OD value of treatment group and control group. To further verify the MTT results, we use Calcein-AM/PI method to stain live and dead cells, respectively. In brief, cells were seeded into a 6-well plate and incubated for 24 h, followed by incubation with CDs, Ce6, CDs-Ce6/LDH for 24 h, respectively. After irradiation with 650 nm light (15 mW/cm^2) for 20 min, the cells were stained with Calcein-AM (5 μg/mL) and PI (10 μg/mL). After a culture for another 20 min and a sufficient washing with PBS, the cells were monitored with an inverted fluorescence microscope.

1.9 Cellular ROS Detection. Hela cells were incubated with CDs, Ce6 (5 μg/mL), and CDs-Ce6/LDH (equivalent 5 μg/mL of Ce6) for 24 h in 6-well plates. After washing with PBS, DCFH-DA (2×10^{-5} M) was added and incubated for 20 min. After irradiation with 650 nm light (15 mW/cm^2) for 20 min, the cells were washed thoroughly with PBS. The generated ROS in cells was quantitatively measured by flow cytometer and fluorescence imaging was performed by a Leica inversed fluorescent microscope.

1.10 Subcellular localization of CDs-Ce6/LDH. Hela cells were cultured into 35 mm confocal dishes and incubated for 24 h. After incubating with CD-Ce6/LDH (equivalent 5 μg/mL of Ce6) for 6 h, the cells were washed with PBS for 3 times sufficiently. The cells were stained with Lyso-Tracker Red (60 nM) and Mito-Tracker Green (50 nM) for 45 min and washed with PBS again for 3 times. A Leica TCS SP8 confocal laser scanning platform was used to capture the fluorescent images.

Sample Characterizations. High resolution Transmission electron microscope (HRTEM) images were recorded with JEOL, JEM-2100; the accelerating voltage was 200 kV. The particle size distribution was determined using a Malvern Mastersizer 2000 laser particle size analyzer. The Fourier transform infrared (FT-IR) spectra were recorded in the range 4000–400 cm^{-1} with 2 cm^{-1} resolution on a Vector 22 (Bruker) spectrophotometer. The UV-vis absorption spectra were collected in the range 250–800 nm on a Shimadzu U-3000 spectrophotometer. Raman measurements were carried out with excitation of 633 nm by using a confocal Raman microspectrometer (Renishaw, inVia-Reflex). The fluorescence spectra were performed on a RF-5301PC fluorospectrophotometer. Fluorescence images of these samples were obtained using Leica TCS SP8 confocal laser scanning platform. XPS spectrum of CDs was investigated *via* a PHIQ2000 X-ray photoelectron spectrometer with Al K X-ray source.



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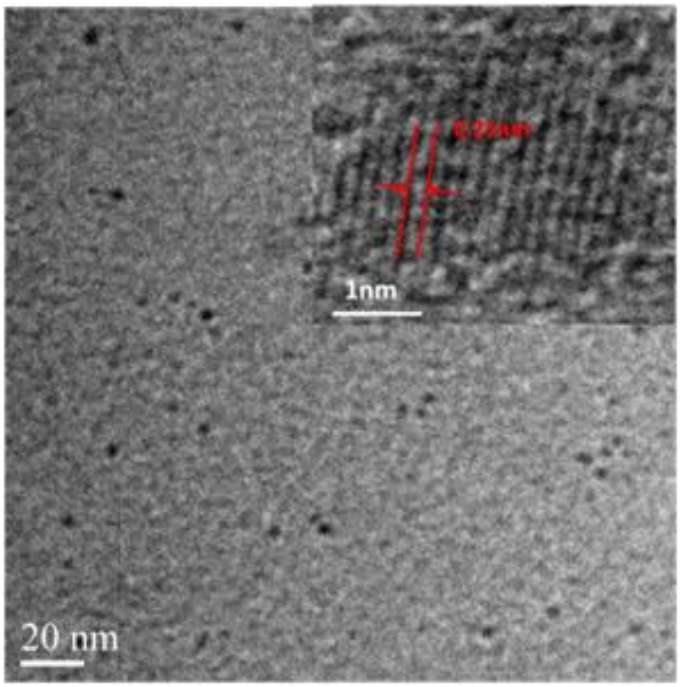


Figure S2. HRTEM image of CDs.

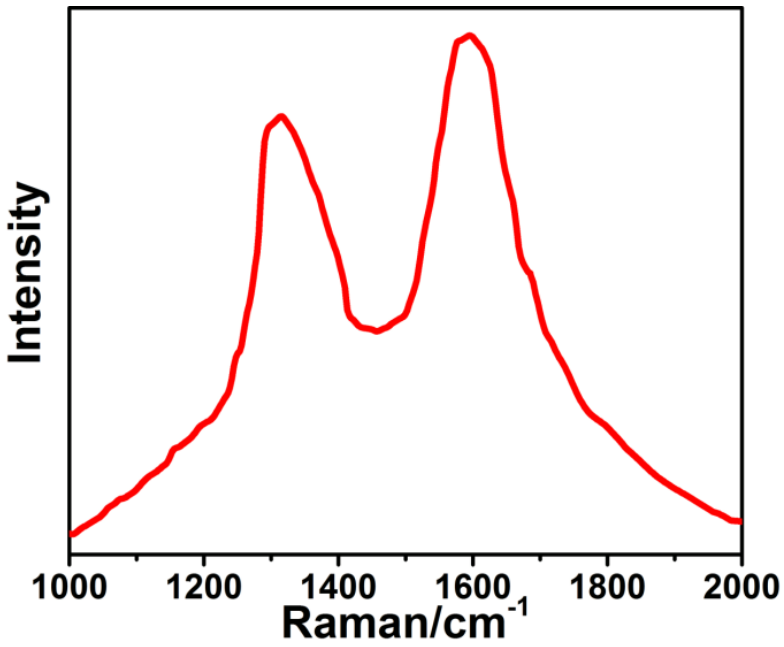


Figure S3. Raman spectrum of CDs.

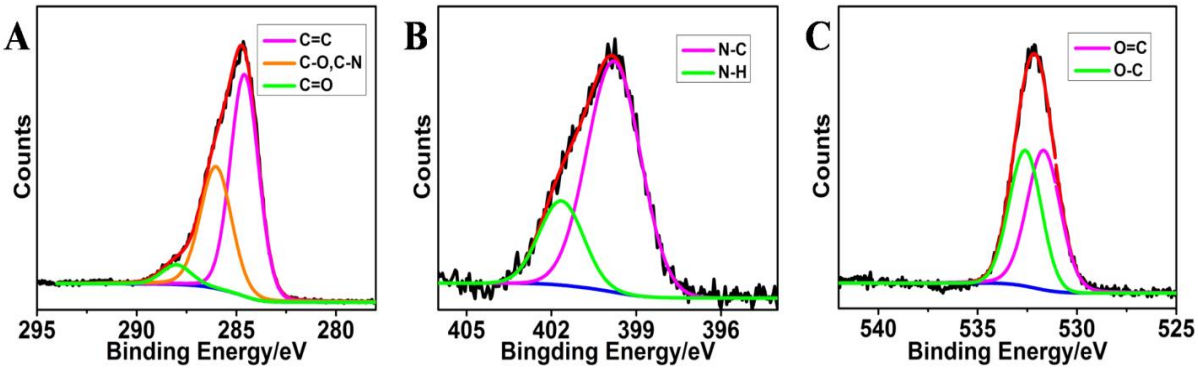


Figure S4. High-resolution XPS spectra of C1*s*, N1*s* and O1*s* for CDs.

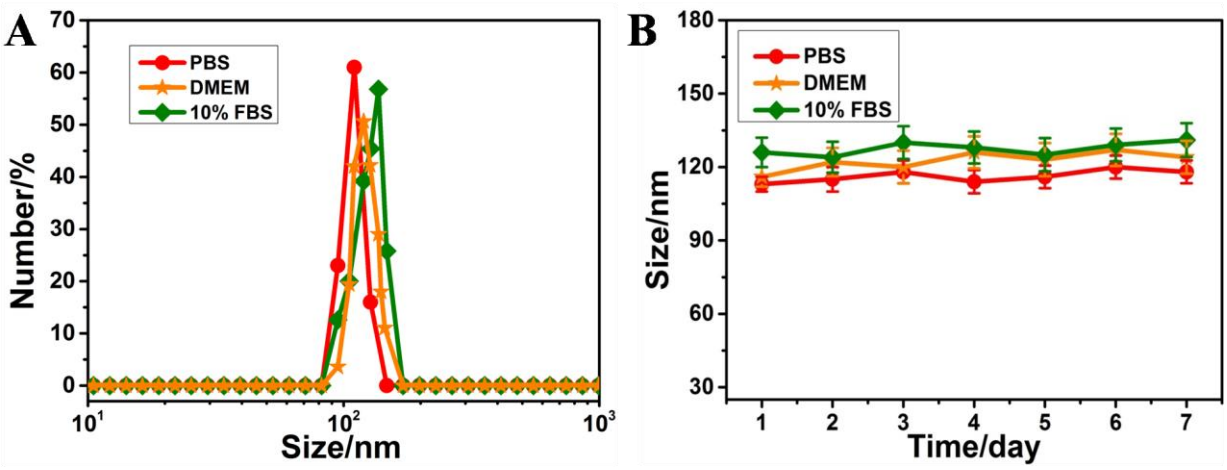
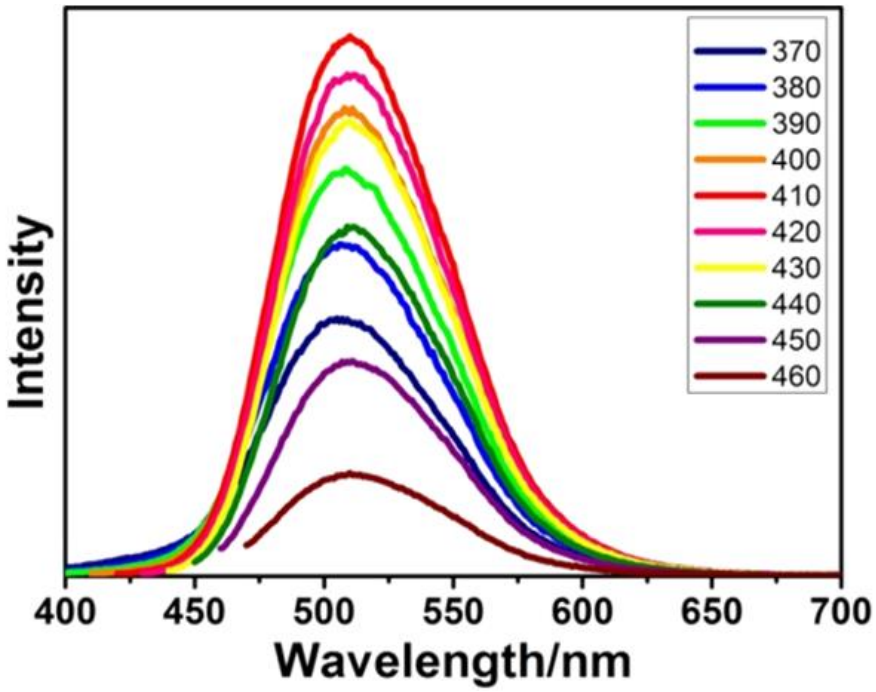


Figure S5. (A) Size distribution of CDs-Ce6/LDH in PBS, DMEM and 10% FBS, respectively. (B) Hydrodynamic diameter of CDs-Ce6/LDH in PBS, DMEM and 10% FBS for one week.



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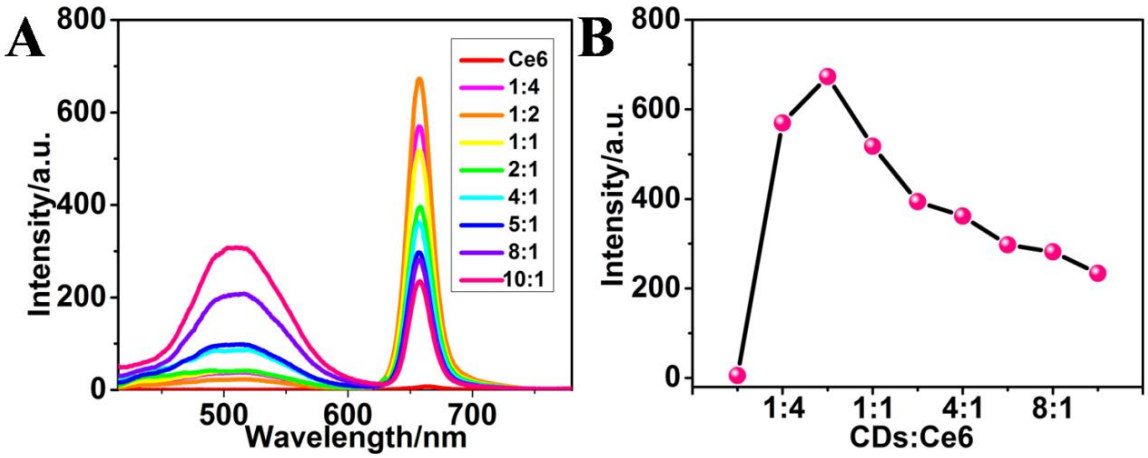

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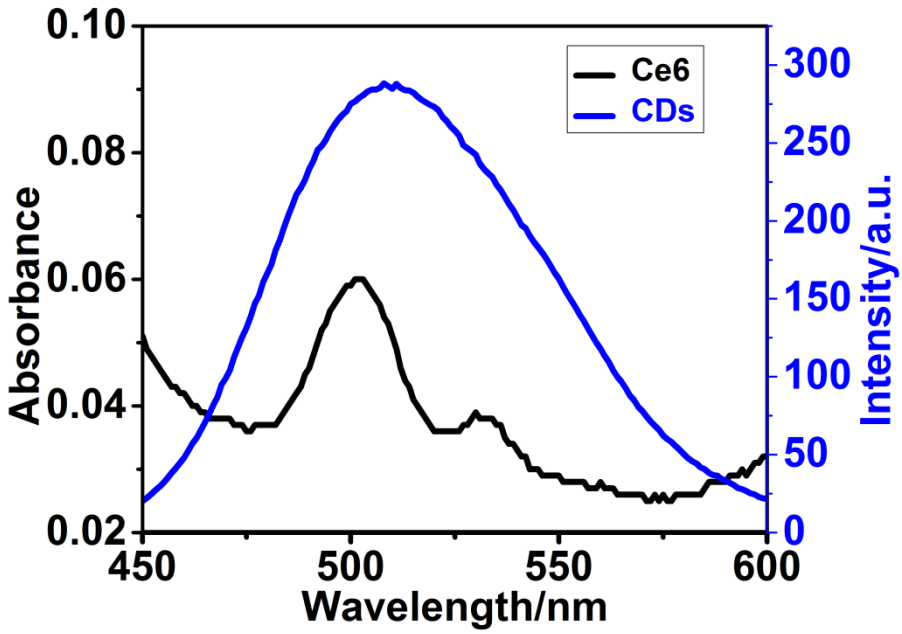
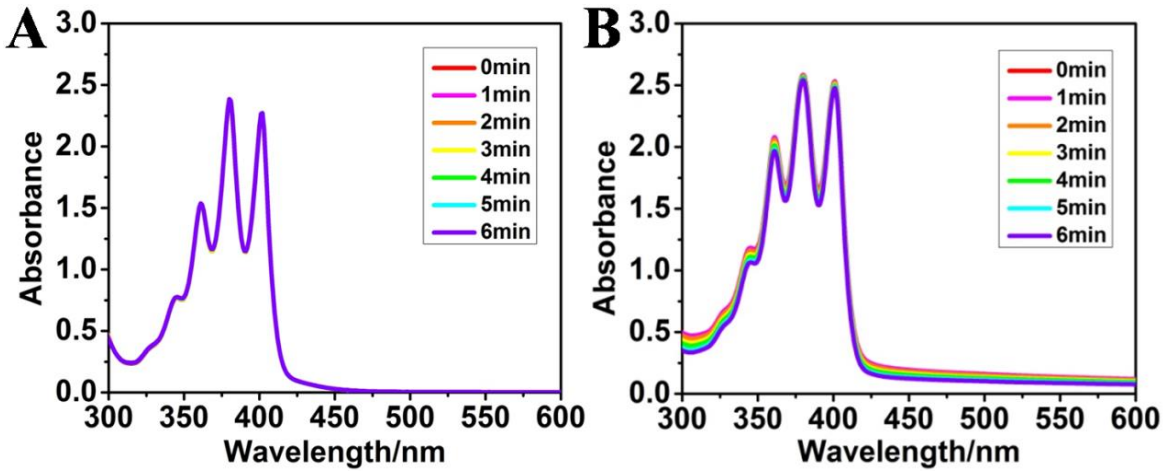


Figure S8. UV-vis absorption spectrum of Ce6 and fluorescence emission spectrum of CDs.



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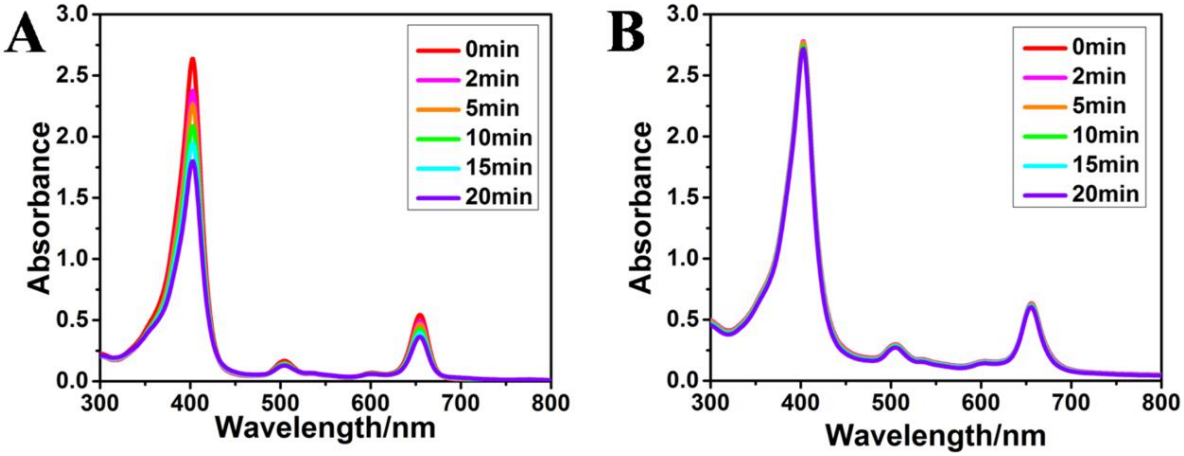

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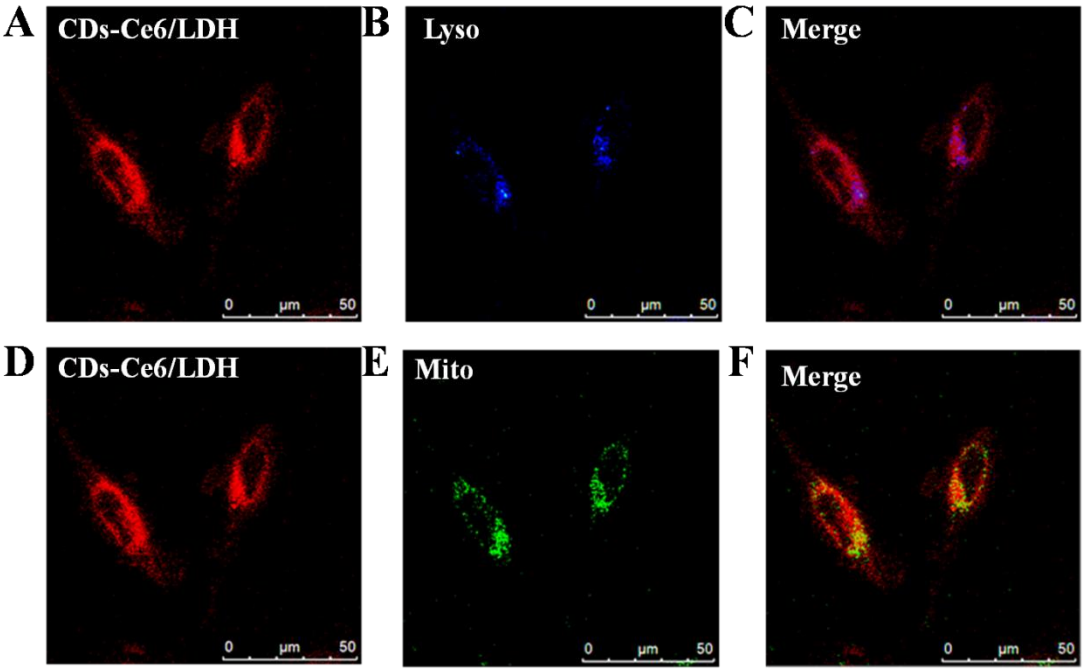

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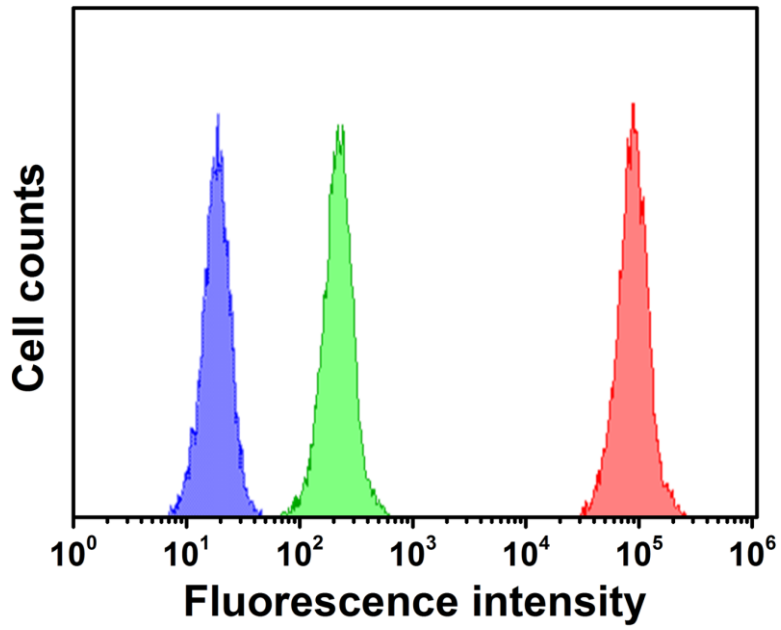

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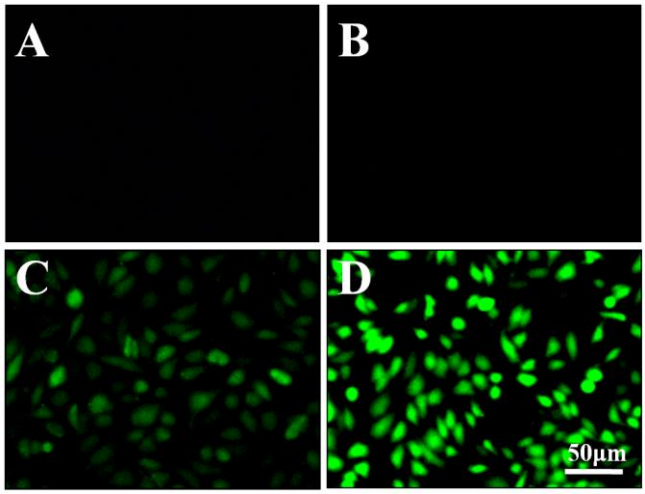

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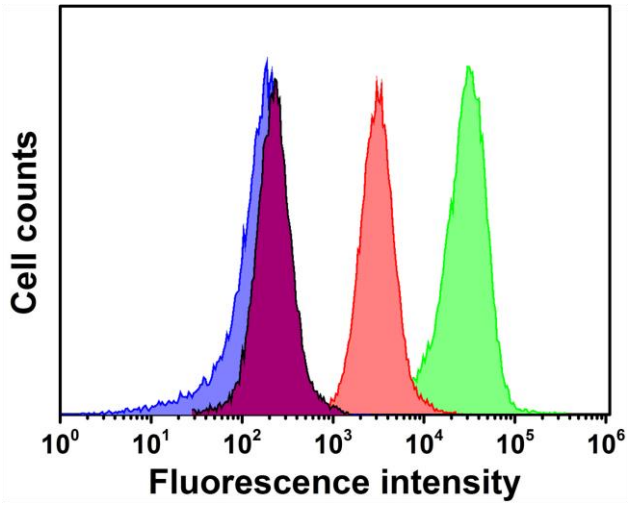
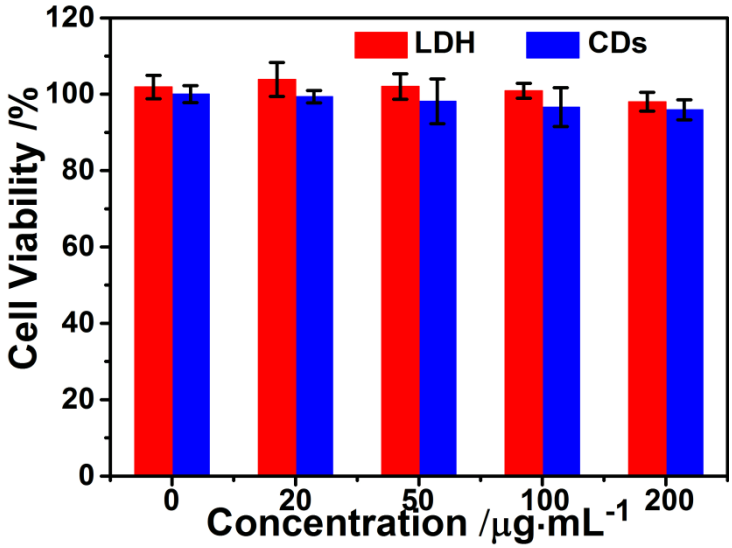


Figure S14. Flow cytometry measurements of DCFH-DA signal for blank (blue), CDs (purple), Ce6 (red) and CD-Ce6/LDH (green), respectively.



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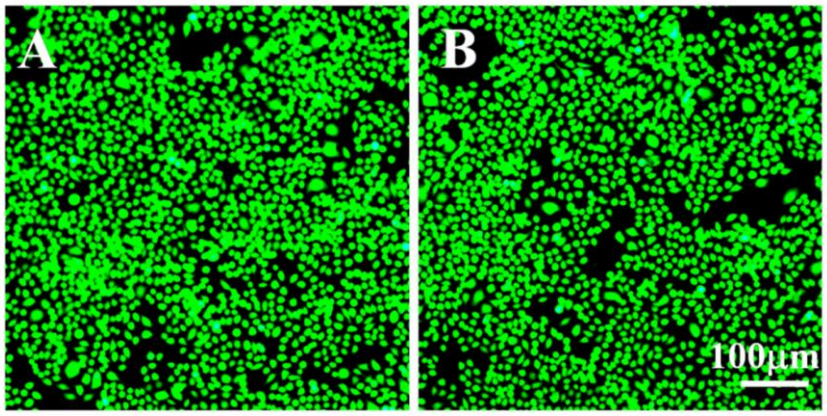

Figure S16. Fluorescence images of Hela cells stained by Calcein AM/PI after treated with (A) CDs and (B) LDH nanosheets at the concentration of 200 μg/mL for 24 h.

References

1. L. Peng, X. Mei, J. He, J. Xu, W. Zhang, R. Liang, M. Wei, D. G. Evans and X. Duan, *Adv. Mater.*, 2018, **30**, 1707389.