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Porphyrin structure carbon dots under red light irradiation for bacterial inactivation

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Electronic Supplementary Information

Experimental details

ROS measurement

1, 3-diphenylisobenzofuran (DPBF) was chosen as ROS trapping agent to indicate the amount of ROS produced. 5.4 mg of DPBF was dissolved into 4 mL of ethanol to form the fresh indicator solution. The ROS measurement was achieved by following the next steps in dark. First, 2 mL with 50 μL of DPBF stock solution was added to the UV cuvette, and covered with seal, then was scanned in UV-Vis absorption spectra recording from 400 to 440 nm. Subsequently, UV-Vis were measured every 1 min under laser irradiation (638-nm, 1 W cm^{-2}) for 20 min. The absorbance at 400–440 nm was recorded. 50 μL of DPBF stock solution was added in 2 mL of DI as control group, and UV-Vis absorption spectra were measured at the same conditions.

Photothermal experiment

To evaluate the photothermal activity, 900 μL of the DI with 100 μL of CDs solution (a final concentration within 25 μg mL^{-1}) was transferred into a 24-well plate. The temperature of the solution (every minute) was recorded under the irradiation during 20 min with a 638-nm laser (0.1-1 W cm^{-2}).

Intracellular ROS detection in bacteria

The intracellular ROS level was measured by CDs mediated PDT using 2',7'-dichlorofluoresce in diacetate (DCFH-DA) through a ROS assay kit (Beyotime Reagent Co., China), according to previous studies[1]. Briefly, *E.coil* (1.0×10^9) were washed three times with PBS and the cell density adjusted to 1.0×10^6 cfu mL^{-1}.

DCFH-DA (10 μM) was added to the bacterial suspension, and then incubated for 20 min at 25°C. The suspensions were washed thoroughly for three times with PBS to remove the DCFH-DA. Then, cleaned bacteria except control were exposed to 1 μg mL^{-1} of CDs for 30 min in dark and irradiated under 638 nm laser (0.05W cm^{-2}) for 3 min. The fluorescence of DCFH-DA was observed under a laser scanning confocal microscope (LSCM) at 488 nm excitation and 525 nm emission wavelengths.

Cytotoxicity experiment

3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS, Promega, (Beijing) Biotech Co., Ltd.) was involved to evaluate the cytotoxicity of CDs, and Bend.3 cells were chosen as model. Briefly, cells were seeded in a 96-well plate (100 cells/well) to adhere for 12 h at 37°C. After that, medium containing different concentrations of CDs (0, 5, 10, 15, 20, 25, 30, 40, 50 μg mL^{-1}) was added. After 24 h of incubation in the dark, the medium was washed thoroughly, and then replaced by 100 μL of fresh medium containing 20 μL of MTS. After incubation for another 1 h, shaking the plate for 3 min. Subsequently, the absorbance at 490 nm was measured using an ELISA plate reader. The cell viability was estimated based on the following equation: cell viability% = $(OD_{CDs} - OD_{control}) / (OD_{cell} - OD_{control})$.

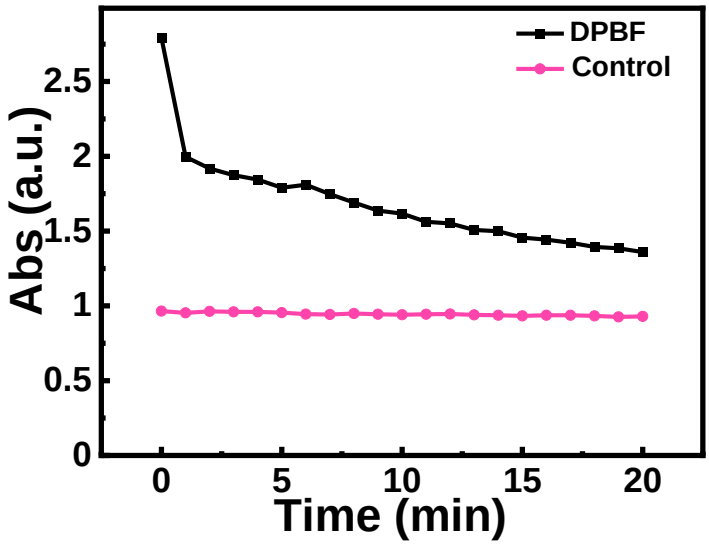

Fig. S1 Absorbance of DPBF at 422 nm under the 638-nm laser irradiation in the presence and absence of 10 μg mL^{-1} CDs as photosensitizer;

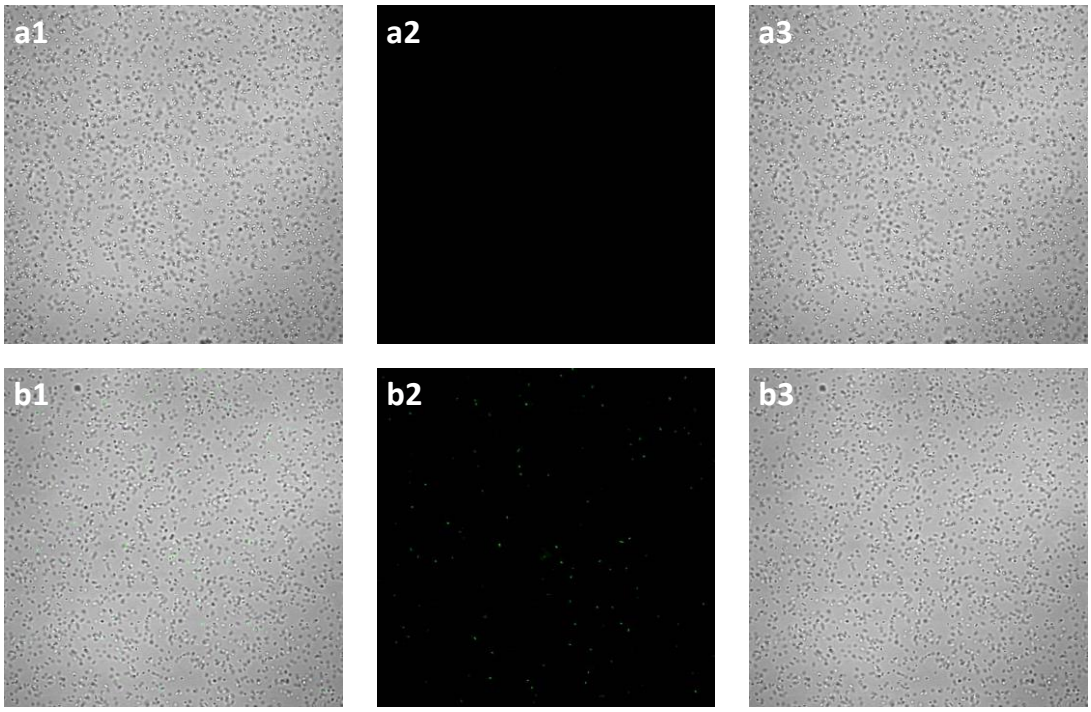

Fig. S2 Intracellular ROS detection in *E.coil* (a1-a3) control group without CDs incubation and (b1-b3) 1 μg mL^{-1} of CDs for 30 min in dark. (a1, b1) merged field, (a2, b2) λ_{ex}=488 nm and λ_{em}=525 nm, (a3, b3) bright field.

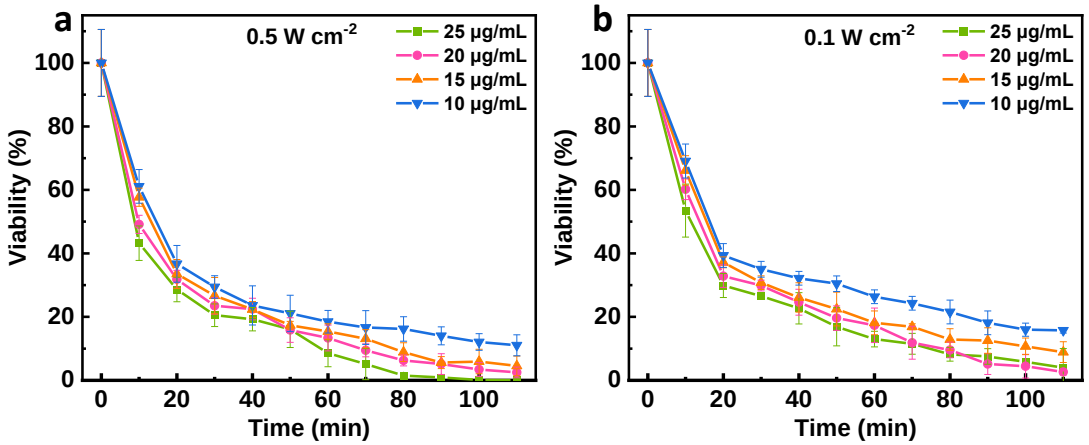

Fig. S3 Within the CDs concentration of 10-25 μg mL^{-1}, the bacteria inactivation by the 638-nm laser with different power densities of (a) 0.5 W cm^{-2} and (b) 0.1 W cm^{-2}.

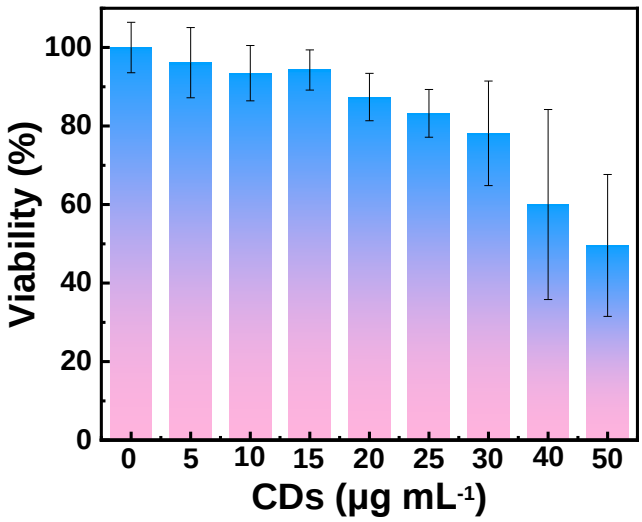

Fig. S4 Cell viability of Bend.3 with CDs (0-50 μg mL^{-1}) in the dark

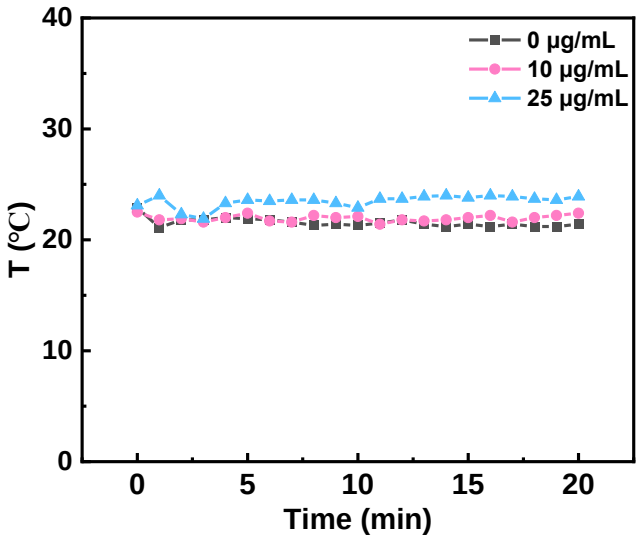

Fig. S5 Temperature measurement with CDs under the 1W cm^{-2} of the 638-nm laser.

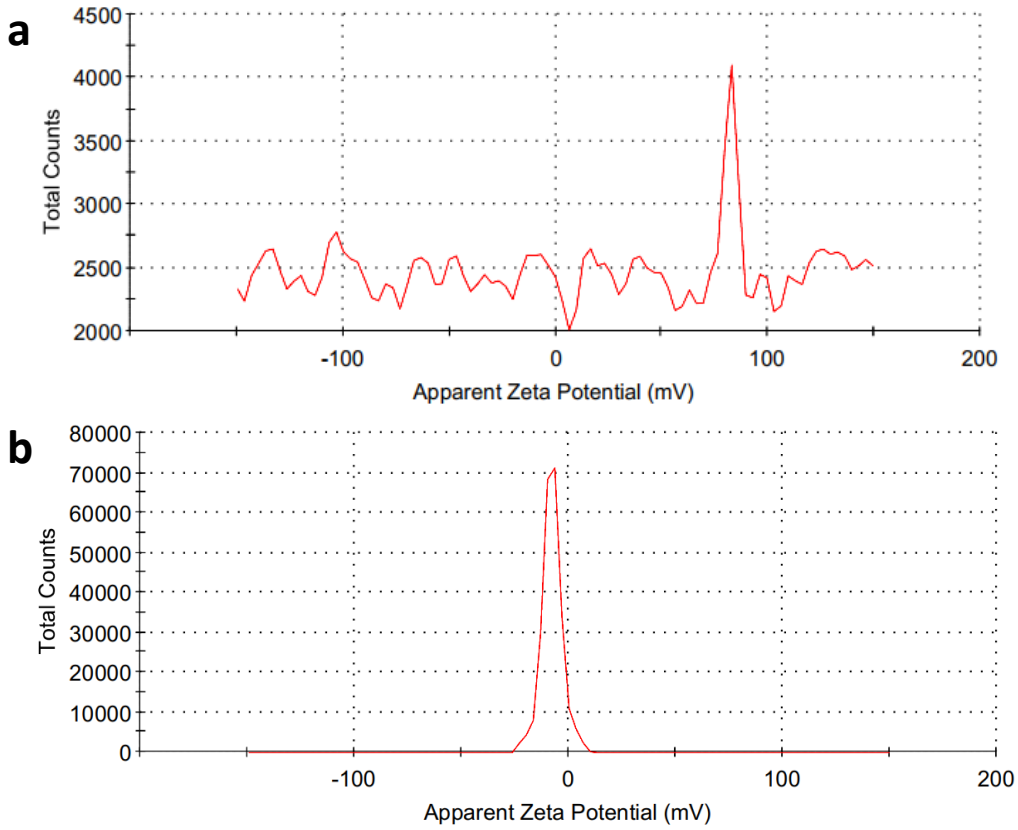

Fig. S6 Zeta potentials of (a) CDs and (b) *E.coil.*

References

1. Dwivedi S, Wahab R, Khan F, et al. Reactive oxygen species mediated bacterial biofilm inhibition via zinc oxide nanoparticles and their statistical determination [J]. PLoS One, 2014, 9(11): e111289.