Supporting Information

Carbon dots-based covalent organic frameworks with high water dispersibility for photodynamic cancer therapy

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Experimental sections

1. Materials and Methods

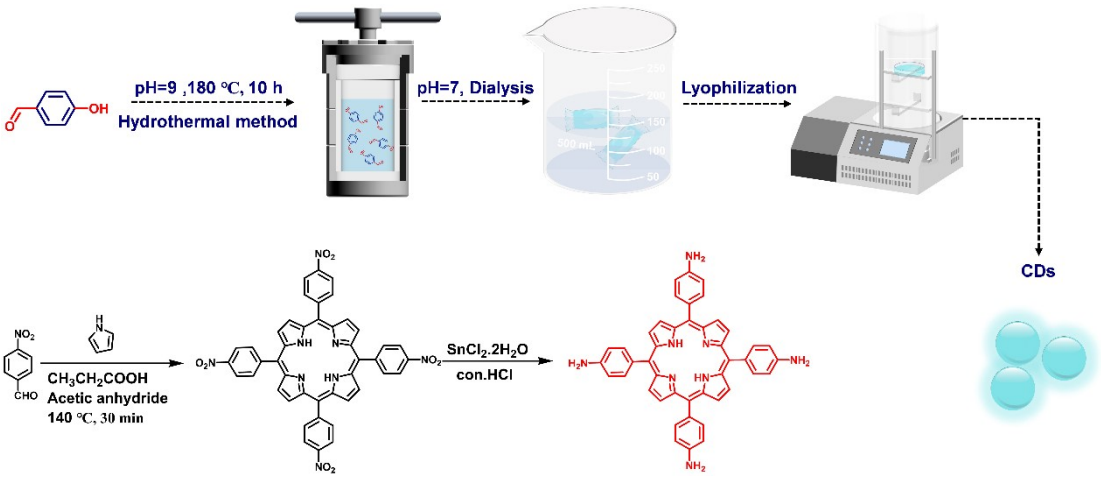
All chemical materials used for the synthesis of CDs-COF were purchased from MACKLIN (China). All the organic solvents were of analytical grade and used without further purification except for the special requirements. ^{1}H NMR was recorded on a Bruker Avance neo 400 spectrometer in $CDCl_3$, using TMS (d = 0.00) as the internal standard. High-resolution mass spectra were obtained on a Bruker Autoflex MALDI-TOF mass spectrometer. Fourier transform infrared (FT-IR) spectra were recorded on a Bomen NICOLET6700 spectrometer. X-ray powder diffraction experiments were carried out on an XRD instrument (D8 ADVANCE X, Bruker, Germany). UV-vis absorption and PL spectrum were collected from shimadzu Model UV-1700 spectrometer and HORIBA Fluoromax-4C-L spectrometer, respectively. Dynamic light scattering (DLS) was measured with Zeta Sizer from Malvern. Transmission electron microscope (TEM) images were obtained using a FEI Talos F200i electron microscope. Nitrogen-adsorption isotherms were measured at 77 K with a

Micromeritics ASAP2020 HD88 Surface Area and Porosity Analyzer, and the BET model was applied for analysis. Calcein AM and propidium iodide (PI) were purchased from J&K Scientific Ltd (China). Fluorescence images of cells were obtained on a Confocal Laser Scanning Fluorescence Microscope (FV1200) from Olympus, Japan.

2. Cell lines

Michigan cancer foundation-7 (MCF-7) cells, Chinese hamster ovary (CHO), and mouse hepatoma (Hepa1-6) cells were obtained from ATCC (Manassas, VA) and cultured in DMEM medium (10% FBS and 1% Penicillin-streptomycin) in a condition of 37 °C and 5% of CO_2.

3. Syntheses of TAPP, CDs and CDs-COF



Scheme S1. Schematic illustration of the syntheses of CDs, TAPP, and CDs-COF.

Synthesis of 5,10,15,20-trakis(4-nitrophenyl)porphyrin

p-nitrobenzaldehyde (11 g, 0.073 mol) was dissolved in 300 mL of propionic acid mixed with 12 mL of acetic anhydride in a round-bottomed flask. The reaction mixture was refluxed at 140 °C for 30 min followed by the addition of 5 mL of freshly distilled pyrrole in 10 mL of propionic acid. After stirring for another 30 min, the resulting product was collected by filtration through a Brinell funnel followed by washing with

water until the filtrate became colorless. The obtained solid was dried and then washed with 80 mL of pyridine followed by refluxing at 120 °C for 1 h to remove residual impurities, affording5,10,15,20-trakis(4-nitrophenyl)porphyrin.

Synthesis of 5,10,15,20-trakis(4-aminophenyl)porphyrin

5,10,15,20-trakis(4-nitrophenyl)porphyrin (1.1 g, 7.4 mmol) was added to a 500 mL reaction flask, followed by the addition of 55 mL of concentrated hydrochloric acid. A solution of $SnCl_2 \cdot 2H_2O$ (4.95 g, 21.9 mmol) in 80 mL of concentrated hydrochloric acid was then added using a dropping funnel. After refluxing at 75 °C for 30 min., 70 mL of ammonia was slowly added under ice water bath, and the protonated impurities were removed by filtration. The resulting powder was added to 200 mL of 5% sodium hydroxide solution and stirred overnight. Purification by Soxhlet extraction with chloroform (80 °C, 2-3 days) afforded TAPP as a dark green solid (254 mg, 27%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.92 (s, 8H), 8.01 (d, J = 7.7 Hz, 8H), 7.09 (d, J = 7.7 Hz, 8H), 4.04 (s, 8H), -2.68 (s, 2H). MALDI-TOF MS: calcd for = 674.2906, found = 674.1656.

Preparation of CDs

4-hydroxybenzaldehyde (24.2 mg, 0.2 mmol) was dispersed ultrasonically in 10 mL of ultrapure water, and the pH of the solution was adjusted to 9 using 0.5 mol/L NaOH. The resulting mixture was transferred to a Teflon-lined stainless-steel autoclave (25 mL) and heated in the oven at 180 °C for 10 h. After cooling to room temperature, the obtained solution was adjusted to neutral pH with dilute hydrochloric acid (HCl) and subsequently dialyzed against deionized water to remove residual impurities.

Synthesis of CDs-COF

a mixture of TAPP (8 mg) and CDs (20 mg) were ultrasonicated in 2 mL of n-butanol for 10 min in a 10 mL pyrex tube, followed by the dropwise addition of 0.2 mL of acetic acid (6 M). The suspension was ultrasonicated for an additional 10 min to disperse all reactants homogeneously. Subsequently, the reaction tube was flash-frozen in liquid nitrogen, degassed through three freeze-pump-thaw cycles, and sealed by butane flame under a vacuum. After heated at 120 ºC for 24 h, the resultant CDs-COF was collected by centrifugation and thoroughly washed with chloroform for 72 h using

Soxhlet extraction. Undergoing 24 h of the drying process, the purified CDs-COF was obtained as a black powder.

4. Experimental methods

ROS generation assay

The ROS generation capacity of CDs-COF was evaluated under 635 nm laser irradiation using 2′,7′-dichlorofluorescin (DCFH) as the ROS probe [1, 2]. Firstly, CDs-COF was dissolved in PBS buffer (pH = 7.4) to a concentration of 80 μg/mL. 3 μL of the above dispersion was then mixed with 3 mL of DCFH (0.5 μM) solution. Upon 635 nm laser (1.4 W/cm^2) irradiation, a time-dependent fluorescence spectrum of the mixed solution was recorded under excitation of 488 nm. The measurement methods for the ROS generation of TAPP and CDs were the same as above.

In vitro degradability of CDs-COF

To study the pH-triggered degradability, 0.9 mg of CDs-COF was firstly dispersed in 3 mL of PBS solution (pH = 7.4 or 5.5) at 37 ºC. At predetermined time intervals(0, 30, 60, 90, 150, 210, and 270 min), the PBS solutions with different pH values were then analyzed by recording the absorbance at 460 nm and the fluorescence intensity at 678 nm (λ_{ex} = 430 nm), respectively. For each condition, the analysis was performedin triplicate.

In vitro cytotoxicity assays

To explore the photodynamic effect of CDs-COF, Hepa1-6 cells were cultured in a 96-well plate (1 × 10^4 cells per well) and subsequently treated with varing concentrations of CDs-COF (0, 10, 20, 30, 50, 70, and 90 μg/mL). After an additional 12 h of incubation, the cells were exposed to 635 nm laser irradiation (0.3 W/cm^2) for 10 min. After 24 h of incubation, a CCK-8 assay was employed to assess the cytotoxicity of CDs-COF [3, 4]. The cell viability was calculated based on the following equation.

$$\text{Cell viability (\%)} = (A_C - A_{blank})/ (A_0 - A_{blank}) \times 100\%$$

Where A_C represents the absorbance value of the cells treated with different

concentrations of CDs-COF, A_0 is the absorbance value of cells without treatment, and A_{blank} is the absorbance value of the bare CCK- 8 medium at 450 nm. All samples were performed in four parallel repeats.

For live/dead cell assay, Hepa1-6 cells were introduced into Petri dishes (3×10^3 cells per well). After incubation with CDs-COF (90 μg/mL) or PBS (pH = 7.4) for 12 h, the cells were treated with 635 nm laser (0.3 W cm^{-2}) for 10 min. With a further 12 h of incubation, the killing effects of CDs-COF on Hepa1-6 cells were determined by Calcein-AM/PI co-staining tests by a fluorescence microscope (OLYMPUS IX73P1F, Japan) [5, 6].

Intracellular ROS detection

DCFH-DA can be deacetylated to DCFH with the presence of lactonase, which was then rapidly oxidized by ROS to yield the green-emissive DCF [7, 8]. Accordingly, a fluorescence microscope (OLYMPUS IX73P1F, Japan) was employed to evaluate the generation of intracellular ROS. Firstly, Hepa1-6 cells were incubated with CDs-COF (90 μg/mL) or PBS in a petri dish for 12 h. After the cells were washed with PBS three times, DCFH-DA (1×10^{-7} mM) was loaded on the cells and further incubated for 30 min. After washing with PBS three times to remove extracellular DCFH-DA, the cells received 635 nm laser irradiation (0.3 W cm^{-2}) for 10 min. Finally, the fluorescence images were captured, and the generation of ROS was indicated by the green fluorescence of DCF [9, 10].

Cellular uptake experiment

To evaluate the cellular uptake of CDs-COF, Hepa1-6 cell lines were cultured in a 96-well plate (1×10^4 cells per well) containing in DMEM medium. The cells were maintained for 24 h at 37 °C. Cells were then treated with CDs-COF at concentration of 50 μg/mL and incubated for 24 h at 37 °C. Afterwards, the cells were washed with phosphate buffer. The cell uptake efficiency of CDs-COF on Hepa1-6 cells were determined by a fluorescence microscope (OLYMPUS IX73P1F, Japan).

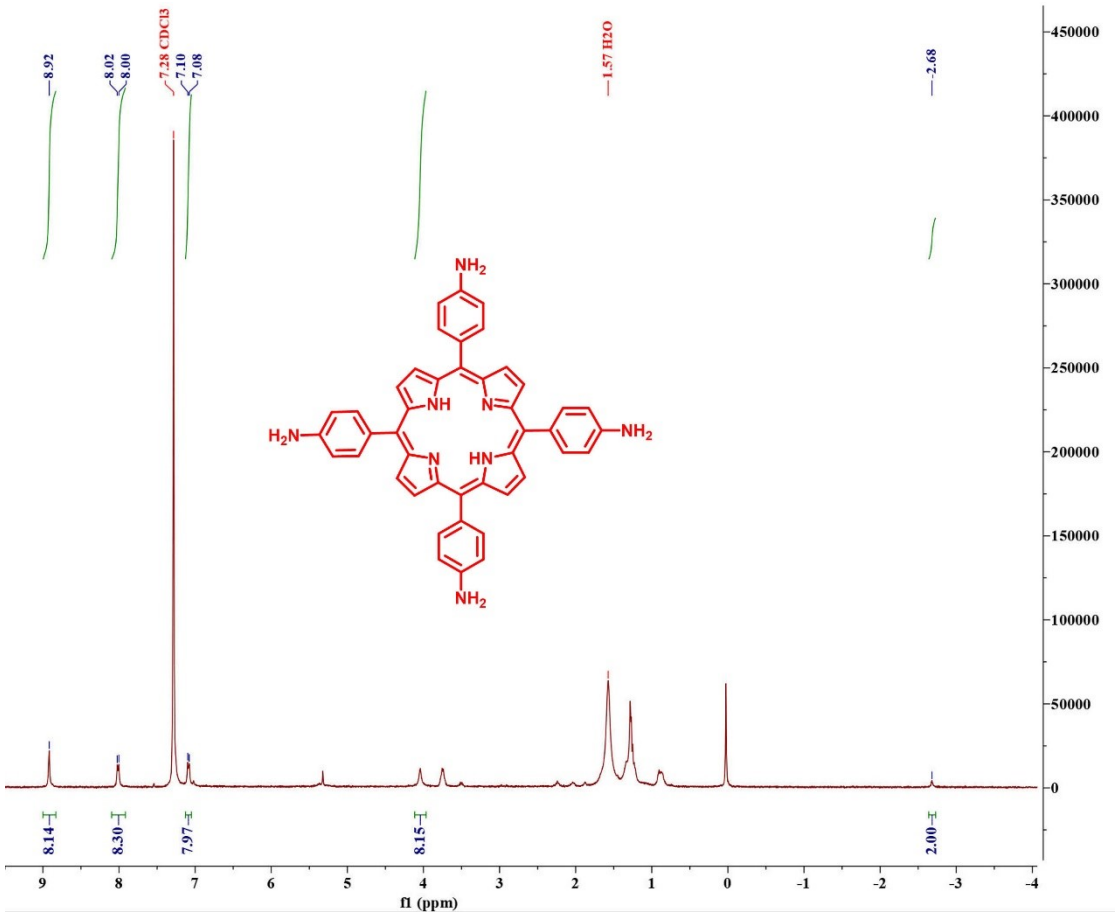


Figure S1. ^{1}H NMR spectrum of TAPP

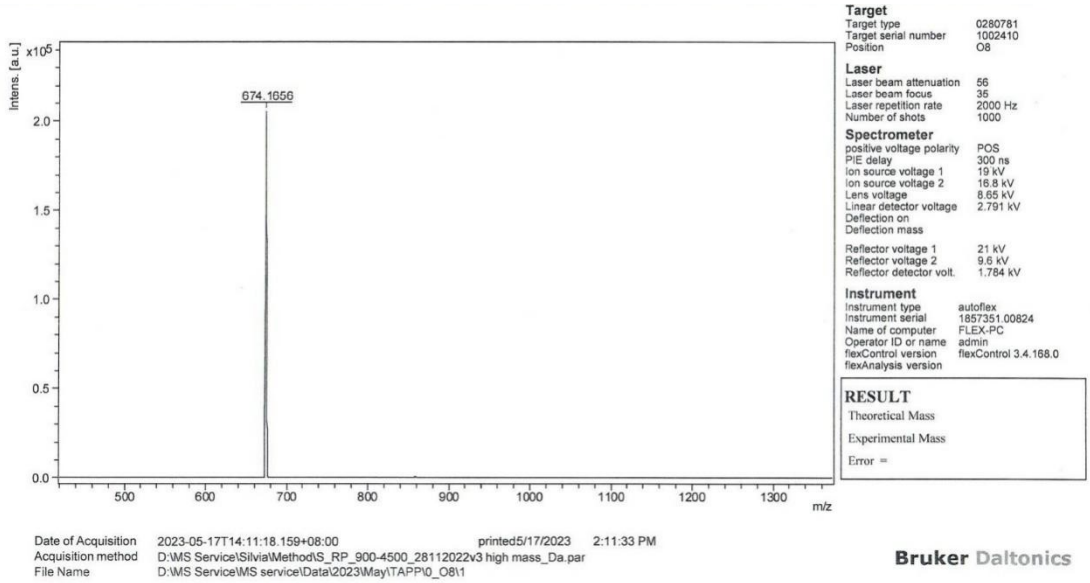


Figure S2. MALDI-TOF-MS spectrum of TAPP

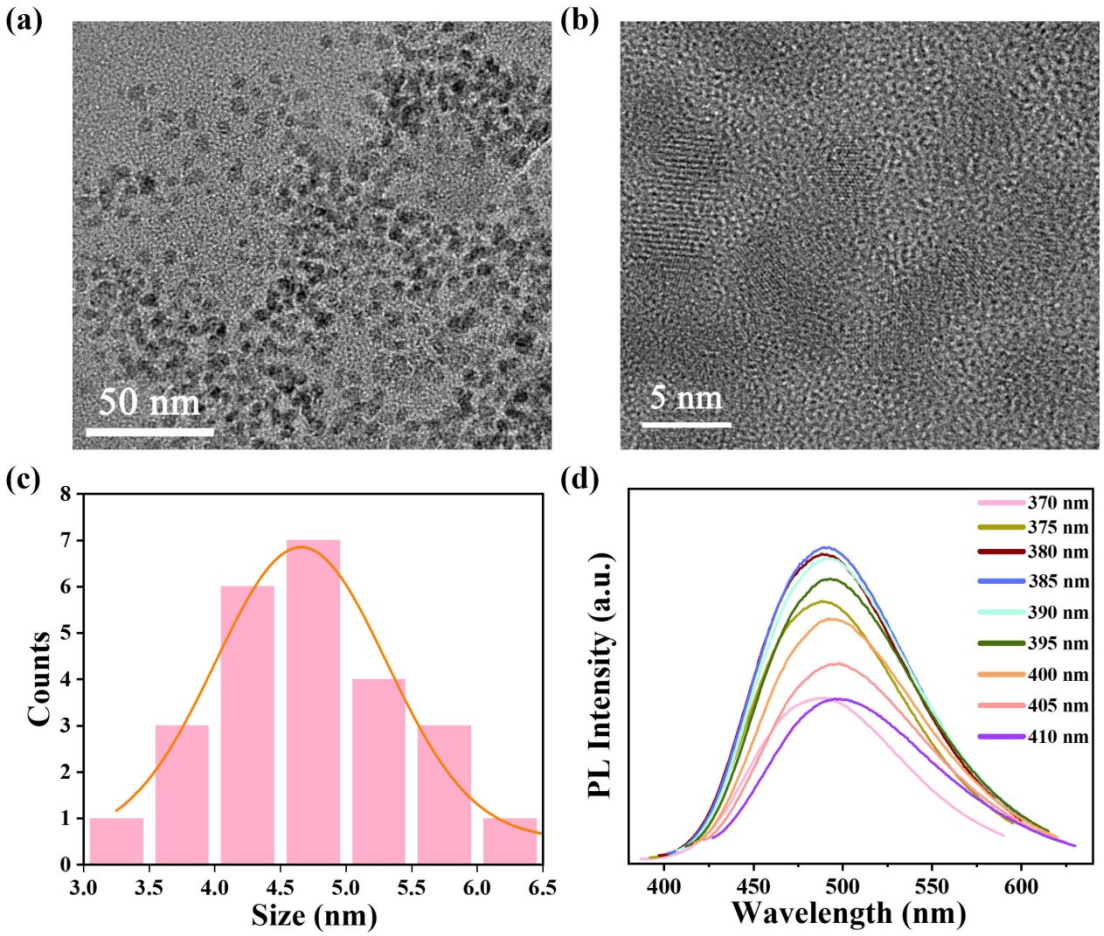


Figure S3. (a, b) TEM image, (c) size distribution, and (d) excitation-dependent PL spectra of CDs.

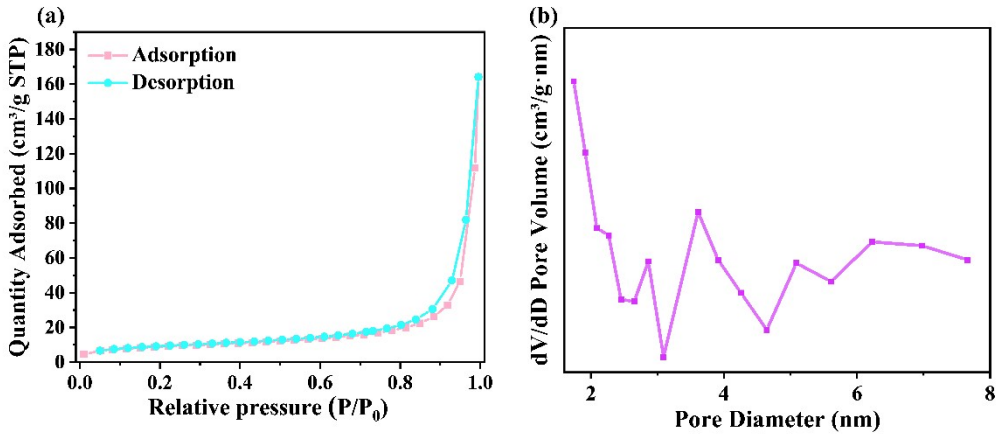


Figure S4. (a) Nitrogen adsorption and desorption isotherms of CDs-COF. (b) Pore size distribution of CDs-COF.

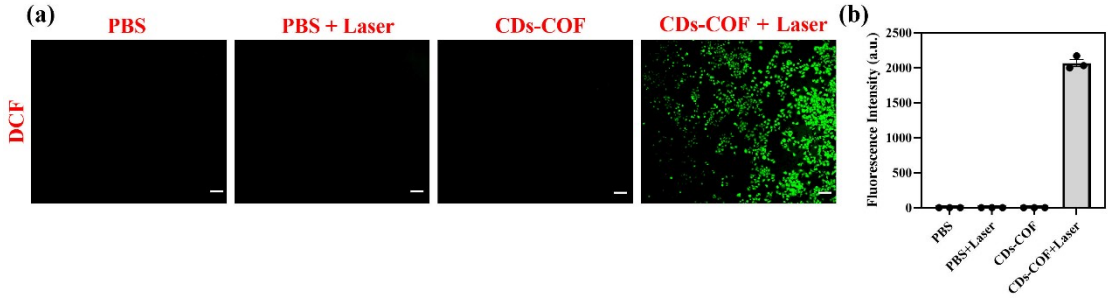


Figure S5. (a) ROS generation in Hepa1-6 cells treated with PBS, PBS + Laser, CDs-COF, or CDs-COF + Laser, as observed under a fluorescence microscope (Scale bar: 100 μm). (b) Statistical data of DCF fluorescence intensities (calculated by Image J, n=3).

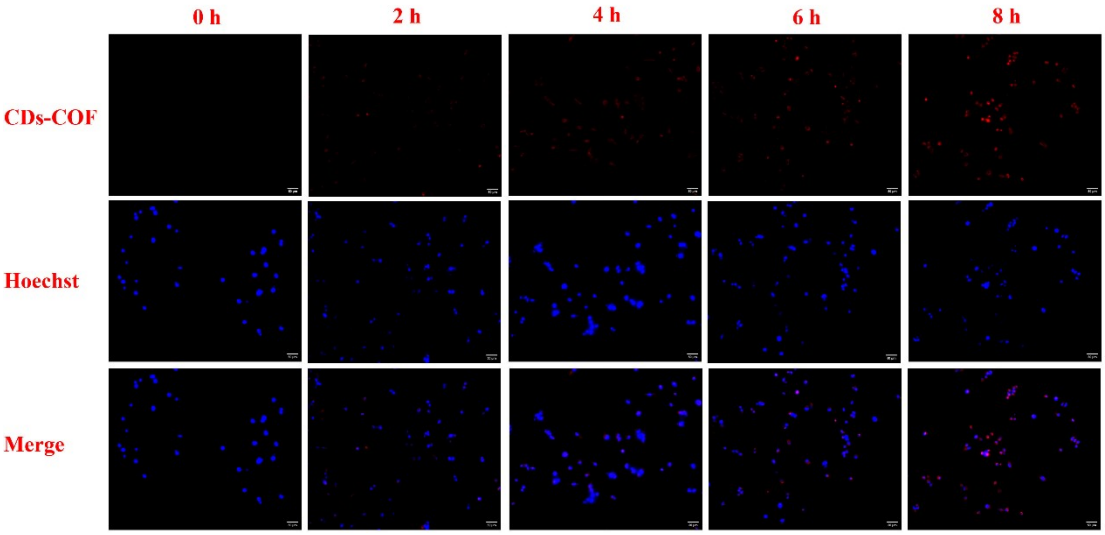


Figure S6. Cellular uptake of CDs-COF in Hepa1-6 cells at the concentration of 50 μg/mL for different times (scale bar = 50 μm).

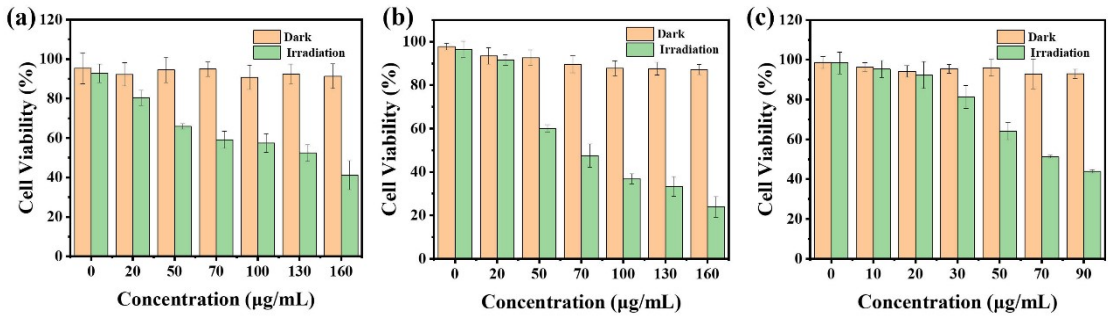


Figure S7. Cell viability of (a) CHO cells and (b) MCF-7 cells treated with CDs-COF, and (c) Hepa1-6 cells treated with TAPP at various concentrations with dark or 635 nm laser irradiation (0.3 W cm^{-2}) for 10 min (n = 5).

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