Electronic Supplementary Material (ESI) for Biomaterials Science.

Supporting Information

Controlled ROS Production by Corannulene: The Vehicle Makes a Difference

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1. Materials

Corannulene (Cor) was kindly provided by Prof. Jay Siegel from Tianjin University. Gama-cyclodextrin (γ-CD) was purchased from Shanghai Yuanye Biology & Technology Co., Ltd. (Shanghai, China). 2',7'-Dichlorofluorescein diacetate (DCFH-DA), and MitoSOX™ Red were from ThermoFisher Scientific (Beijing, China). Methoxy-poly(ethylene glycol) alkyne (mPEG-Alkyne, Mw = 1000 Da) was sourced from XingJiaFeng Science & Technology Development Co., Ltd. (Shenzhen, China). Dihydroethidium (DHE) was sourced from Beyotime Biotechnology (Beijing, China). Hydrogen peroxide assay kit was purchased from Abcam (Shanghai, China). All other agents were acquired from Jiangtian Chemicals (Tianjin, China). Milli-Q water was used throughout the study.

2. Preparation of γ-CD/Cor complex and mPEG-Cor micelles

In brief, an aqueous solution of γ-CD (622.6 mg, 0.48 mmol, 3 mL) was mixed with a solution of Cor (40.0 mg, 0.16 mmol) in DMSO (1.5 mL). After 24 h's reaction at 60^{o}C, the system was cooled to room temperature (25^{o}C), followed by multiple water and acetonitrile washing to get the γ-CD/Cor inclusion complex.[S1] The preparation of mPEG-Cor conjugate employed our recently reported method.[S2] Briefly, Cor (0.50 g, 2.0 mmol) and $FeCl_3$ (32.50 mg, 0.20 mmol) was dissolved in 3 mL dichloromethane (DCM) at -78^{o}C. Bromine (0.38 g, 2.4 mmol) was also dissolved in 20 mL DCM (20 mL) which was carefully transferred to the Cor solution. After 6 h, the mixture was supplemented with a saturated aqueous solution of $Na_2S_2O_3$, followed by water washing, $MgSO_4$ treatment, filtration, DCM removal, and column chromatography purification to get Cor-Br. mPEG-Alkyne (120.0 mg, 0.12 mmol), $PdCl_2(PPh_3)_2$ (3.3 mg, 0.005 mmol), and CuI (2.0 mg, 0.011 mmol) were transferred to the mixed solvent containing 1,2-dichloroethane (1 mL) and triethylamine (2 mL). Then COR-Br (32.9 mg, 0.10 mmol) was added dropwise to the above solution. The reaction was maintained at 80°C under nitrogen atmosphere. After 24 h, the crude product was purified by column chromatography with a mixture of ethyl acetate and methanol (9:1, v/v) as the eluent to get mPEG-Cor. The preparation of mPEG-Cor micelles employed a typical dialysis method. [S2]

3 Controlled ROS production *in vitro*

NaOH (10 mM) was used to treat ROS-sensitive DCFH-DA for 30 min to break the ester bond and activate the probe. Afterwards, the above solution was transferred to a series of aqueous solution of either γ-CD/Cor complexes or mPEG-Cor micelles (10, 20, 40 and 60 μM) in PBS (pH 7.2, 25 mM) and the DCF concentration was kept at 5.0 μM. The samples were irradiated (365 nm, 95 mW/cm^2) for different time and the fluorescence signal was collected accordingly (E_x = 485 nm, E_m = 530 nm).

4 ROS type determination

The production of superoxide by γ-CD/Cor complexes and mPEG-Cor micelles was analyzed via using the fluorescent DHE probe that can freely permeate cell membrane. The probe concentration was fixed at 5 μM; the Cor concentration in both samples was also set at 5 μM. The Cor samples were irradiated at 365 nm (95 mW/cm^2) for up to 15 min prior to fluorescence signal collection (Ex/Em = 510/610 nm) (n = 3). To verify the generation of hydrogen peroxide by γ-CD/Cor complexes and mPEG-Cor micelles, the typical hydrogen peroxide assay kit that utilized the reaction between hydrogen peroxide and OxiRed probe to produce fluorescent signal in the presence of horse radish peroxidase (HRP) (Ex/Em = 535/587 nm). The Cor concentration was also at 5 μM (n = 3). Electron spin resonance (ESR) spectroscopy was employed to investigate the formation of hydroxyl radical by γ-CD/Cor complexes or mPEG-Cor micelles. In brief, 60 μL γ-CD/Cor (50 μM) or mPEG/Cor (50 μM) aqueous solution was mixed with 40 μL 5,5-Dimethyl-pyrroline-oxide (DMPO) that was used as the spin-trapping agent. Both samples were irradiated at 365 nm (95 mW/cm^2) for 30 min prior to collect the ESR signal of DMPO-OH.

5 Intracellular ROS detection and mitochondrial ROS detection

Human prostate cancer (PC-3) cells were kindly provided by Prof. Dan Ding from Nankai University. The PC-3 cells were seeded in 35-mm plates at a density of 1×10^4 cells per well and grown in RPMI 1640 medium containing 10% fetal bovine serum and 100 U/mL penicillin–streptomycin. The cell culture was maintained at 37^{o}C in a humidified atmosphere containing 5% CO_2 for 24 h. Afterwards, the medium was then replaced with 1 ml of fresh medium containing either γ-CD/Cor complexes or mPEG-Cor micelles (100 μM). After 3 h, the cells were washed by PBS in triplicate and supplemented with

DCFH-DA (20 μM) dissolved in the medium (200 μL). Half an hour later, light irradiation was applied (365 nm, 95 mW/cm^2) for 20 min. The images were recorded by a laser scanning confocal microscope (Zeiss. LSM710) (E_x = 488 nm, E_m = 520-600 nm). Placebo cells were used as the control. Mitochondrial detection of ROS employed the same procedure, but a different MitoSOX probe (5 μM) in HBSS (200 μL) was used (E_x = 514 nm, E_m = 550-600 nm).

6 Cell viability and cellular uptake

PC-3 cells were chosen and the standard MTT method was employed for cell viability analysis. The cells at a density of 4,000 cells/well were incubated with a range of γ-CD/Cor complexes or mEPG-Cor micelles differing in concentration. The light irradiation condition was 365 nm and 95 mW/cm^2 at different time course (0, 5, 10 and 15 min). The cell viability was determined 24 h post light irradiation. At a specific lighting condition, the viability of cells were plotted against Cor concentration (n = 3). The extent to which Cor complexes or micelles were internalized by PC-3 cells was examined by high performance liquid chromatography (HPLC). In brief, the cells were seeded at a density of 10,000 cells/well in 12-well plates, and then incubated with either γ-CD/Cor complexes or mPEG-Cor micelles (100 μM). Three hours later, the medium was discarded and the cells were washed by PBS in triplicate. Thereafter, the cells were digested by trypsin followed by sonication treatment and centrifugation (5,000 × *g*). The supernatant was diluted with acetonitrile before Cor quantification by HPLC. The separation was achieved by a Phenomenex Gemini C18 column (250 mm × 4.6 mm, 5 μm) at 30^{o}C. The mobile phase was acetonitrile with an injection volume of 20 μL and flow rate of 1 mL/min. A UV detector was employed and the wavelength was 263 nm (mPEG-Cor) and 253 nm (γ-CD/Cor), respectively. The mass ratio of internalized and supplemented Cor was calculated to assess the degree of Cor uptake by cells (n = 3).

4. Reference

[S1] S. Liu, D. Lu, X. Wang, D. Ding, D. Kong, Z. Wang and Y. Zhao, J. Mater. Chem. B, 2017 (in press, doi: 10.1039/C7TB00954B).

[S2] X. Dong, X. Guo, G. Liu, A. Fan, Z. Wang, Y. Zhao, Chem. Commun. 2017, 53, 3822.

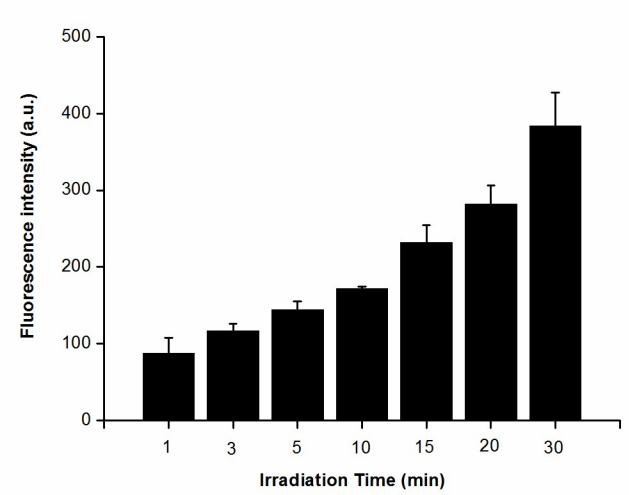
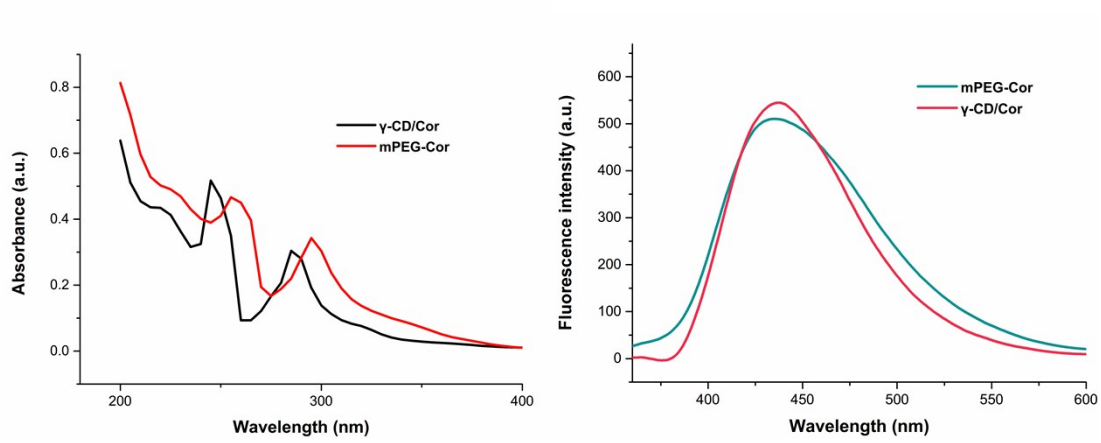
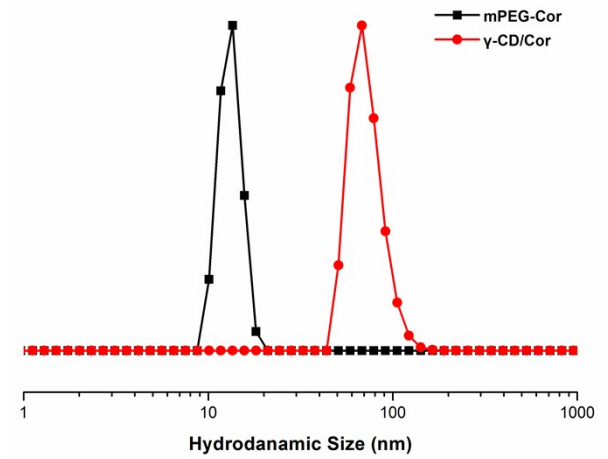


Figure S1. The fluorescence of activated DCFH-DA probe (5 μM) in phosphate buffer saline (pH 7.2, 25 mM,) upon the laser irradiation at 365 nm for up to 30 min (n = 3). The excitation and emission wavelength for probe fluorescence detection was 485 nm and 530 nm, respectively.

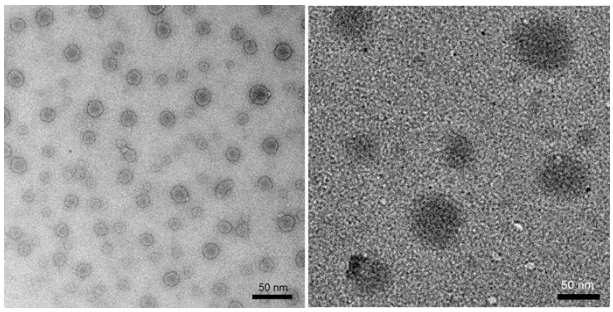


(A) **(B)**

Figure S2. (A) Absorption spectra of methoxy poly(ethylene glycol)-corannulene (mPEG-Cor) and gama-cyclodextrin-corannulene complex (γ-CD/Cor) in water. The Cor concentration was 15 μM for both samples; (B) Emission spectra of mPEG-Cor and γ-CD/Cor in water (5 μM). The excitation wavelength was 290 nm.

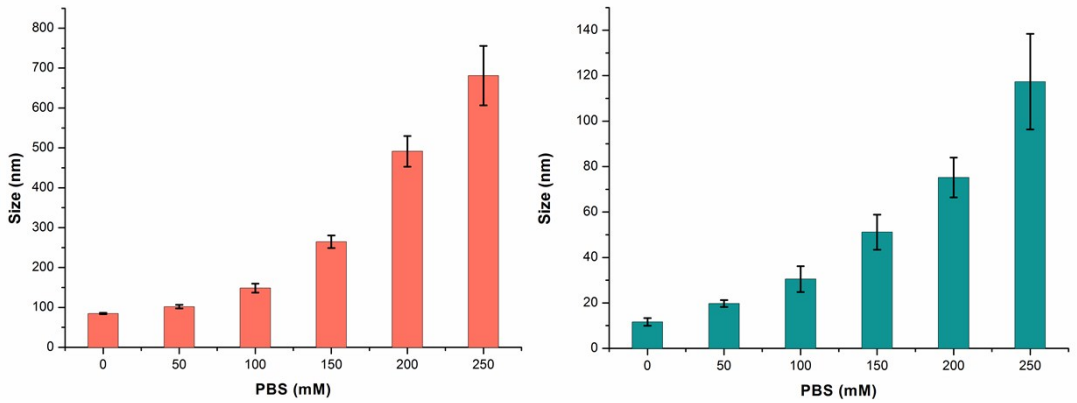


(A)



(B)

Figure S3. (A) The hydrodynamic diameter of methoxy poly(ethylene glycol)-corannulene (mPEG-Cor) and gama-cyclodextrin-corannulene complex (γ-CD/Cor); (B) Transmission electron microscope (TEM) images of mPEG-Cor (*left*) and γ-CD/Cor (*right*).



(A) **(B)**

Figure S4. Stability of gama-cyclodextrin-corannulene complex (γ-CD/Cor) (A) and methoxy poly(ethylene glycol)-corannulene (mPEG-Cor) (B). Data were presented as the hydrodynamic size of formulations in phosphate buffered saline with different ionic strength (n = 3).

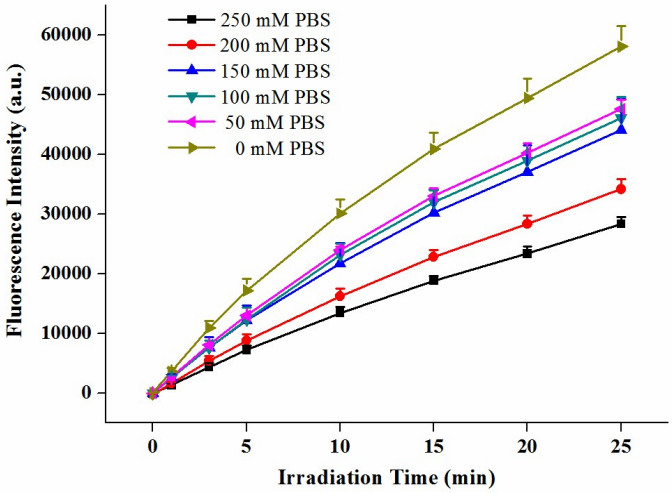


Figure S5. The influence of PBS ionic strength on the ROS generation capability of methoxy poly(ethylene glycol)-corannulene (mPEG-Cor). Data were presented as the fluorescence intensity of DCFH-DA probe (n = 3).

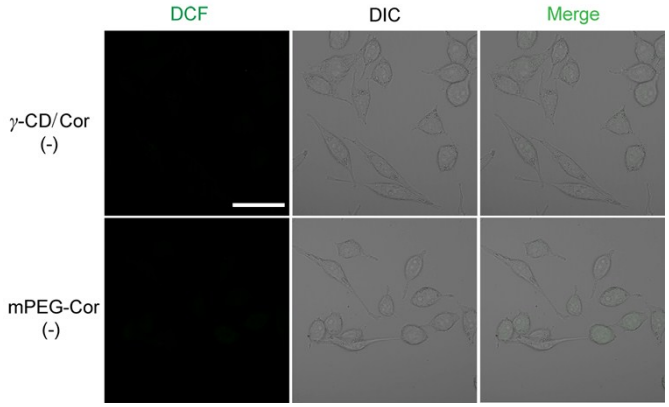


Figure S6. Confocal laser scanning microscope images of PC-3 cells treated by either γ-CD/Cor complexes or mPEG-Cor micelles with placebo cells as control. The symbol "-" indicates the absence of any light irradiation. DCF is the activated ROS-sensitive fluorescent probe (20 μM); DIC represents differential interference contrast. Excitation: 488 nm, Emission: 520-600 nm (scale bar: 50 μm).

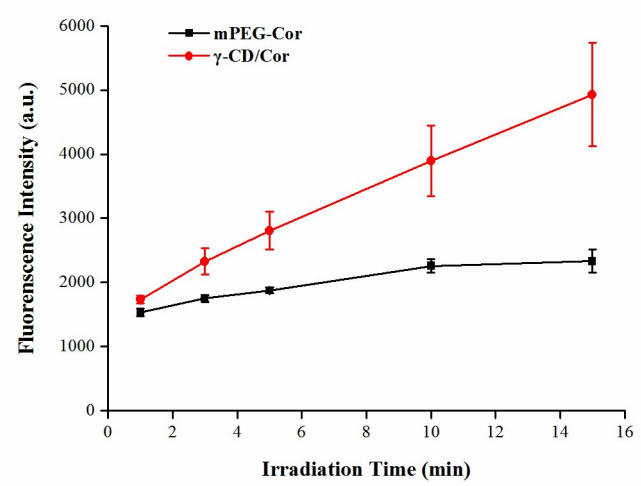


Figure S7. Controlled production of superoxide by methoxy poly(ethylene glycol)-corannulene (mPEG-Cor) and gama-cyclodextrin-corannulene complex (γ-CD/Cor). The superoxide-specific dihydroethidium probe was employed at a concentration 5 μM. The Cor concentration was set at 5 μM (n = 3).

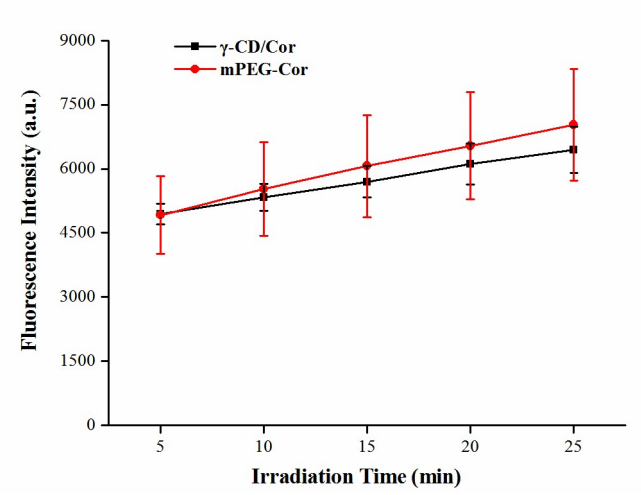


Figure S8. Controlled production of hydrogen peroxide by methoxy poly(ethylene glycol)-corannulene (mPEG-Cor) and gama-cyclodextrin-corannulene complex (γ-CD/Cor). The Cor concentration was set at 5 μM (n = 3). The hydrogen peroxide assay kit was employed for the assay based on the standard protocol.

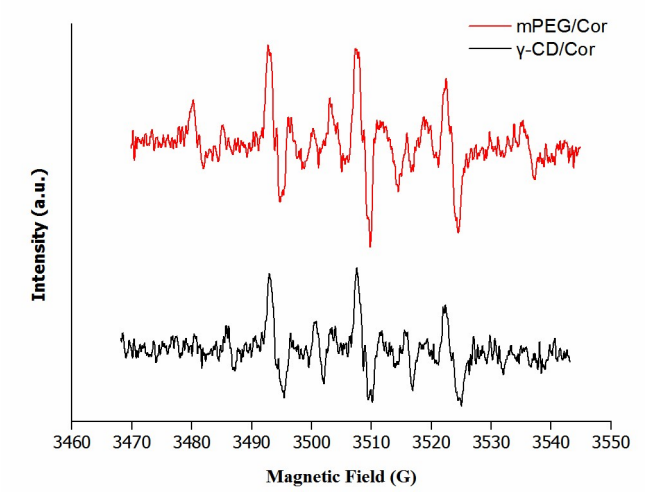


Figure S9. Electron spin resonance (ESR) spectra of methoxy poly(ethylene glycol)-corannulene (mPEG-Cor) and gama-cyclodextrin-corannulene complex (γ-CD/Cor). 5,5-Dimethyl-pyrroline-oxide (DMPO) was used as the probe.

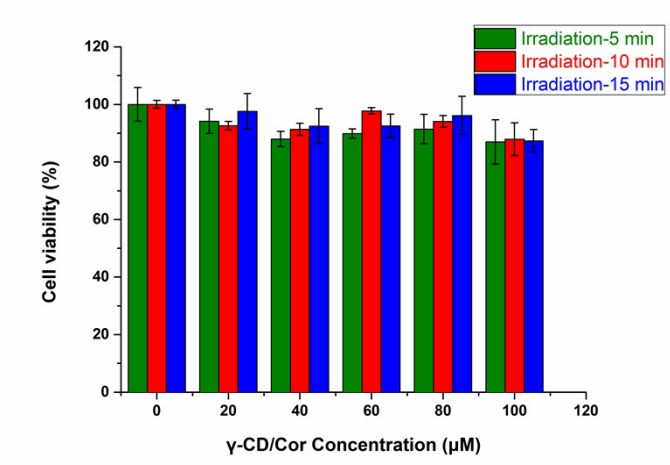


Figure S10. The dose-dependent viability of PC-3 cells in response to γ-CD/Cor complexes and the cells were first treated by light irradiation (365 nm, 95 mW/cm^2) before complex supplementation.

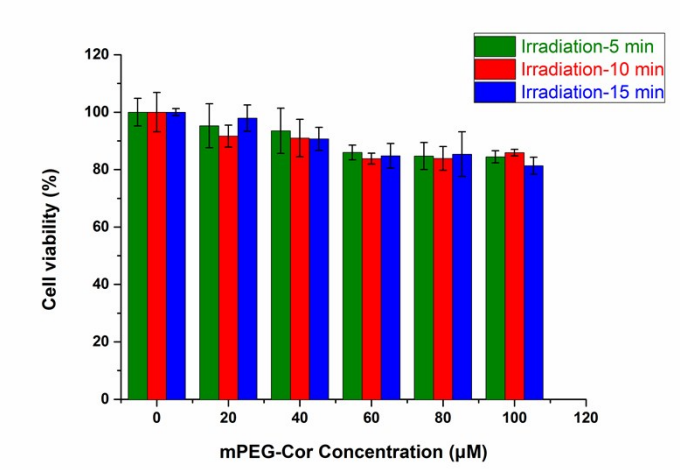


Figure S11. The dose-dependent viability of PC-3 cells in response to mPEG-Cor micelles and the cells were first treated by light irradiation (365 nm, 95 mW/cm^2) before micelles supplementation.

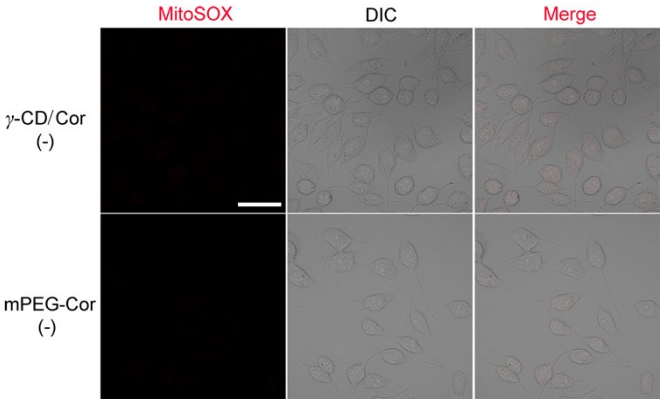


Figure S12. Confocal laser scanning microscope images of PC-3 cells treated by either γ-CD/Cor complexes or mPEG-Cor micelles with placebo cells as control. The symbol "-" indicates the absence of any light irradiation. MitoSOX is the mitochondria-specific fluorescent probe (5 μM); DIC represents differential interference contrast. Excitation: 514 nm, Emission: 550-600 nm (scale bar: 50 μm).