Supporting Information

A DNA origami nanostructure embedded with NQO1-activated prodrugs for precision drug delivery

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Experimental section

Materials. All DNA staple strands with ULTRAPAGE purification were purchased from Sangon (Shanghai, China). M13 DNA was bought from BIORULER (Jiangsu, China). N-hydroxysuccinimide (NHS), 4-(N,N-dimethylamino)pyridine (DMAP), Dox, N,N'-dicyclohexylcarbodiimide (DCC), dichloromethane (DCM), 3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)butanoic acid, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltet- razolium bromide (MTT) and reduced nicotinamide adenine dinucleotide (NADH) were provided by Aladdin (Shanghai, China). Recombinant NQO1 was bought from Abcam (UK). Fetal bovine serum (FBS) was supplied by Gibco (USA). All the chemicals were applied without further purification. Dulbecco's Modified Eagle Medium (DMEM), Trypsin–EDTA (0.25%) and Honchest 33342 was purchased from KeyGen Biotech Co. Ltd. (Nanjing, China). Ultrapure water with resistivity of 18.2 MΩ cm was prepared by a Milli-Q system from Millipore (USA).

Apparatus and characterization. The ^{1}H-NMR characterization was operated on a Bruker DPX 400 MHz spectrometer. The UV–Vis absorption spectra were acquired by a UV-3600 spectrophotometer (Shimadzu, Japan). The fluorescence spectrum was collected by a RF-5301PC fluorophotometer (Shimadzu, Japan). The zeta potential and hydrodynamic diameter were measured on a ZetaPALS analyzer (Brookhaven, USA). The polyacrylamide gel electrophoresis (PAGE) and agarose gel electrophoresis were conducted with an electrophoresis analyzer (Bio-Rad, USA) and observed on a MINI Space system (Tanon, China). MTT assay was performed on a CMax Plus microplate reader (Molecular Devices, USA). Confocal laser scanning microscopy (CLSM) images were acquired using an Olympus FV3000 confocal fluorescence microscope (Leica,

German).

Synthesis of PDox and NQO1-catalyzed drug recovery

As displayed in Fig. S1a in the Electronic Supplementary Material (ESM), 3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)butanoic acid was synthesized according to a previous literature.[1] Dox (1.0 g) was then mixed with the obtained 3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)butanoic acid at a 1:1 molar ratio in 40 mL of anhydrous DMF under constant stirring. N,N-Diisopropylethylamine (2.0 eq) was added dropwise to the mixture during which deep red color appeared. The mixture was stirred at room temperature under nitrogen atmosphere. Thin layer chromatography was applied to monitor the progress of the reaction. After that, the mixture was evaporated under reduced pressure and the residual solution was purified by column chromatography to collect PDox.

To demonstrate the NQO1-stimulated drug recovery of PDox, 50 μg mL^{-1} PDox was incubated with NQO1 (100 ng mL^{-1}) along with NADH (300 mM) in 1 × PBS buffer at 37 °C overnight. The sample was then analyzed using nanospray desorption electrospray ionization mass spectrometry.

Self-assembly of rectangular DNA origami

Rectangular DNA origami was assembled according to previously reported procedures with slight modifications.[2] The side staples were excluded to avoid stacking of adjacent DNA origami. In this experiment, M13 DNA scaffold strand (2.5 nM) and DNA staple strands (25.0 nM) were mixed in 1 × TAE-Mg^{2+} buffer (40.0 mM Tris base, 20.0 mM HAc, 2.0 mM EDTA, and 12.5 mM $MgCl_2$, pH 8.0). The above mixture was then placed in a thermal cycler for programmed annealing from 95 ^{o}C to 25 ^{o}C over 2 h. The DNA origami was ultrafiltered with 100 kDa (MWCO) centrifuge filters followed by three times of washing with 1 × TAE-Mg^{2+} buffer to remove the redundant DNA staples. Finally, the purified product was collected and stored at 4 ^{o}C for further application.

PDox loading into origami

In this experiment, EtOH was introduced to improve the dispersion of hydrophobic PDox to favor cargo loading. In detail, three volume of DNA origami in 1 × TAE with 16.5 mM $MgCl_2$ and one volume of PDox in EtOH were mixed in microcentrifuge tubes at different molar ratios. In the control group, the experimental conditions were the same as above except that DNA origami was not added. After gently shaking overnight, the caps of the centrifuge tubes were opened to allow the EtOH to evaporate. During this process, uninserted PDox settled to the bottom of the tubes owing to the hydrophobicity of PDox, while the resultant PDox@origami remained in the supernatant. The precipitated PDox was isolated by centrifugation (2,000 rpm, 10 min), redispersed in ethanol solution (25%) and quantified by a UV–Vis spectrophotometer. The loading capacity of DNA origami was then calculated based on the standard calibration curve of PDox in

ethanol solution (25%). To assess the prodrug release from PDox@origami, 200 μL of PDox@origami was added into Slide-A-LyzerTM MINI dialysis units (MWCO: 10 kDa) against 1.3 mL of 1 × PBS buffer containing 12.5 mM $MgCl_2$ at pH 7.4 or 5.0. At predetermined time points, 100 μL of the solution was periodically pipetted from the tube and the same volume of fresh buffer was replenished. The PDox release rate was then determined based on the characteristic absorbance of PDox at 502 nm.

Gel electrophoresis

To assess the stability of DNA nanovehicle, DNA origami was exposed to 10% FBS for different time (0, 3, 8, 12, 14 and 24 h) and then analyzed by 1% agarose gel electrophoresis. In order to highlight the enzymatic resistance of DNA origami, one of the staple strands was selected as the control to receive the same treatment and analyzed by 8% PAGE gel imaging.

Cell culture

L02 and MCF-7 cells were supplied by KeyGen Biotech Co. Ltd. (Nanjing, China) and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS, 100 μg mL^{-1} streptomycin and 100 U mL^{-1} penicillin. The cells were cultured in a humidified incubator with 5% CO_2 supply at 37 ^oC.

Cellular internalization of PDox@origami

To study the cellular uptake behavior of PDox@origami, MCF-7 cells plated on confocal dishes were treated with PDox@origami for different time (6, 18 and 24 h). To further get insight into the internalization mechanisms of PDox@origami, chemical inhibitors were adopted to suppress the corresponding endocytic pathways. Briefly, MCF-7 cells were preincubated with serum-free DMEM medium containing 225.0 mM sucrose, 25.0 μg mL^{-1} genistein and 1.0 mM amiloride, respectively. After 30 min of incubation, the cells were subsequently cultured with fresh DMEM medium containing PDox@origami for another 1 h in the cell incubator. Prior to CLSM analysis, the above cells receiving different treatments were stained with a nuclear labeling dye (hoechst 33342) for 30 min in the cell incubator and fixed with 4% paraformaldehyde. After washing with sterile PBS, the cells were subjected to CLSM observation.

In vitro cytotoxicity test

MCF-7 cells were seeded in 96-well plates at a density of 5,000/well for 24 h to allow cell adherence, and then exposed to different formulations for 48 h. Afterward, 10 μL of MTT (5.0 mg mL^{-1}) in DMEM medium was added to each well followed by 4 h of culture in the cell incubator. Finally, the medium in each well was discarded and supplemented with 100 μL of DMSO to dissolve the resultant formazan crystals. The cell survival rate was determined based on the optical

absorbance at 492 nm with a background correction at 630 nm as recorded by a microplate reader.

Supplementary Figures

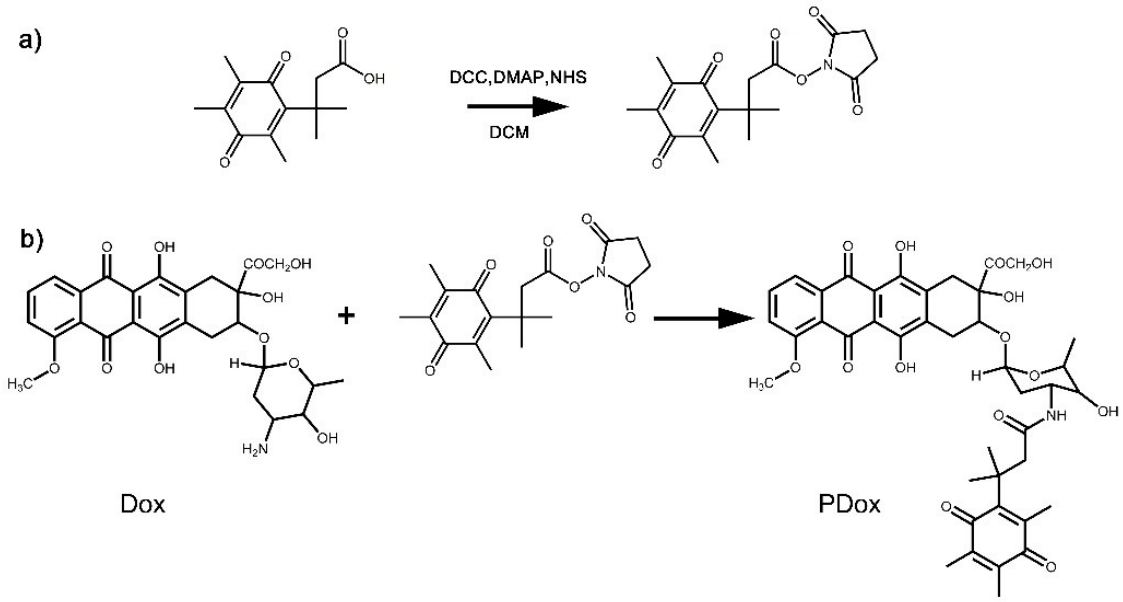

Fig. S1 Synthetic route of PDox.

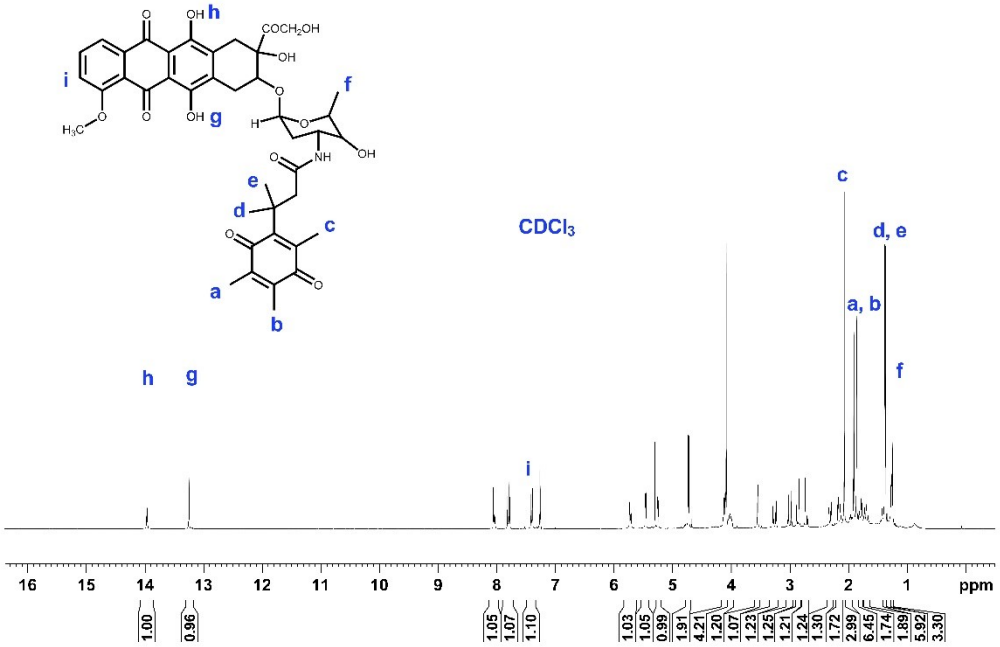

Fig. S2 ^{1}H NMR spectrum of PDox.

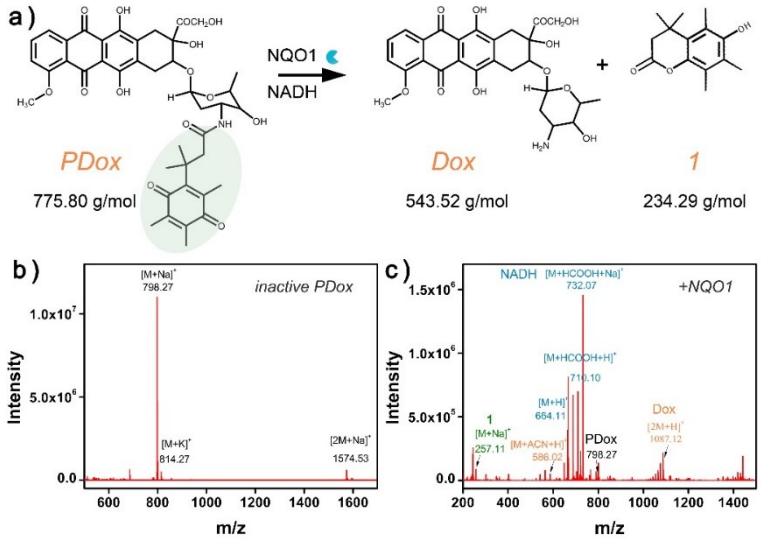

Fig. S3 (a) Schematic representation of NQO1-activated drug recovery. Mass spectrometry analysis of PDox (b) before and (c) after incubation with NQO1. 6-hydroxy-4,4,5,7,8-pentamethyl-2-chromanone was denoted as 1.

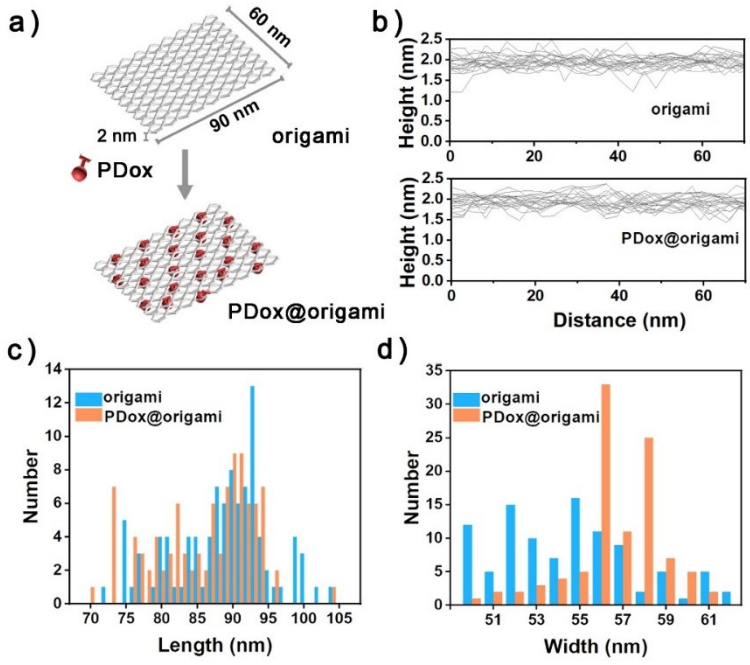

Fig. S4 (a) Schematic illustration showing the process of prodrug loading. The comparison of (b) height, (c) length and (d) width between origami and PDox@origami. In this statistical analysis, the length/width distribution of 100 origamis and the height distribution of 20 origamis were documented by Image J.

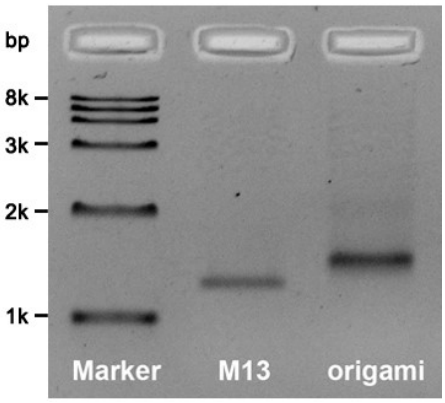

Fig. S5 Agarose gel imaging of M13 scaffold and DNA origami.

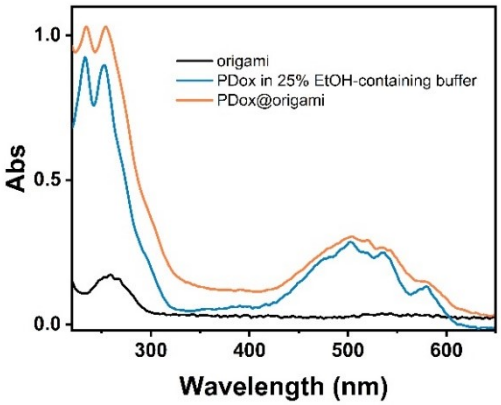

Fig. S6 UV–Vis absorption spectra of origami, free PDox and PDox@origami. Particularly, hydrophobic PDox was dissolved in 25% EtOH-containing buffer for measurement.

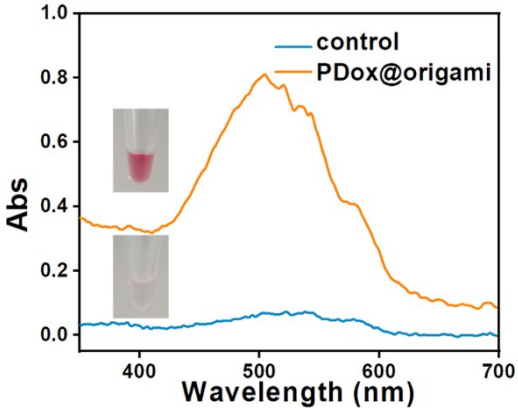

Fig. S7 UV–Vis spectra of the supernatant of PDox solution with or without addition of DNA origami after EtOH evaporation. With the removal of EtOH, free hydrophobic PDox precipitated while PDox@origami remained in the supernatant. Insets are the corresponding photographs.

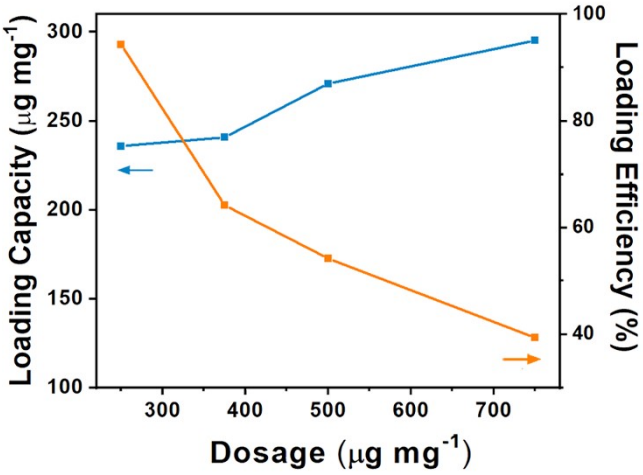

Fig. S8 Loading capacity and loading efficiency curves of DNA origami for PDox.

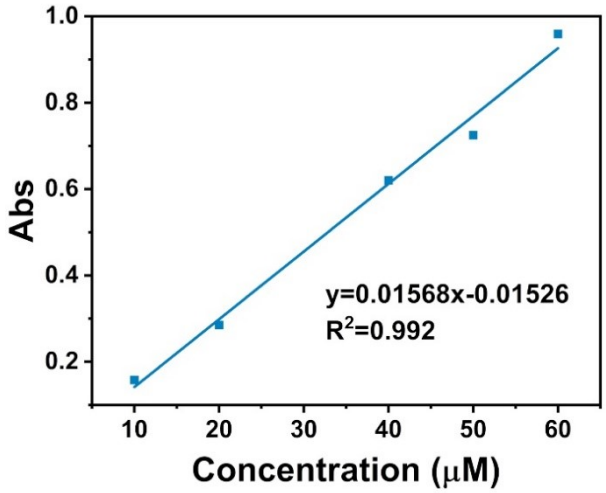

Fig. S9 Standard calibration curve of PDox in 25% EtOH-containing buffer.

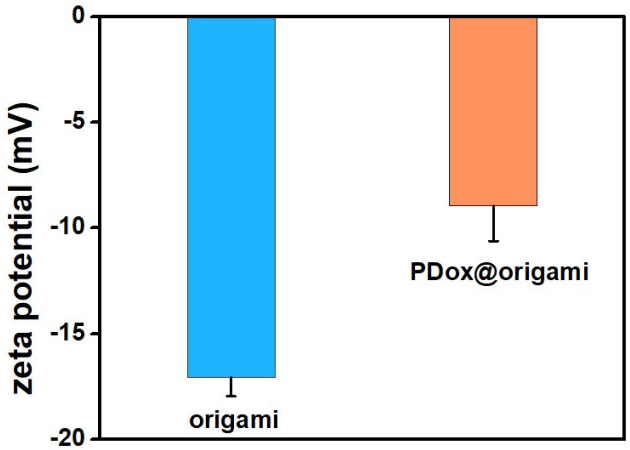

Fig. S10 Zeta potential of DNA origami before and after loading of PDox. Error bars represent the standard deviation (n = 3).

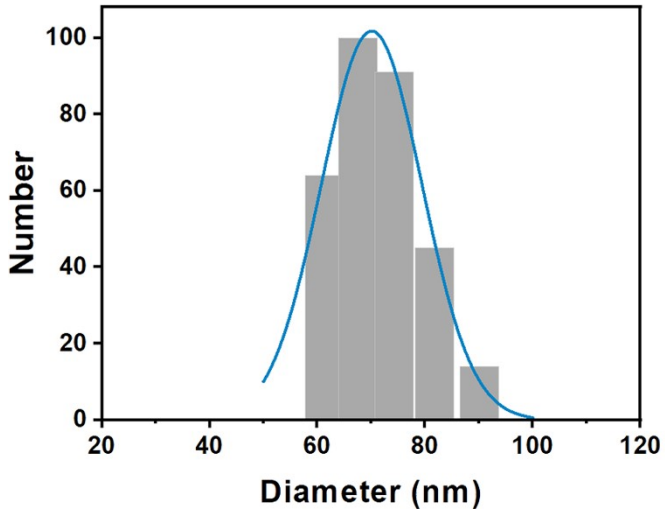

Fig. S11 Hydrodynamic diameter distribution of the formed PDox@origami.

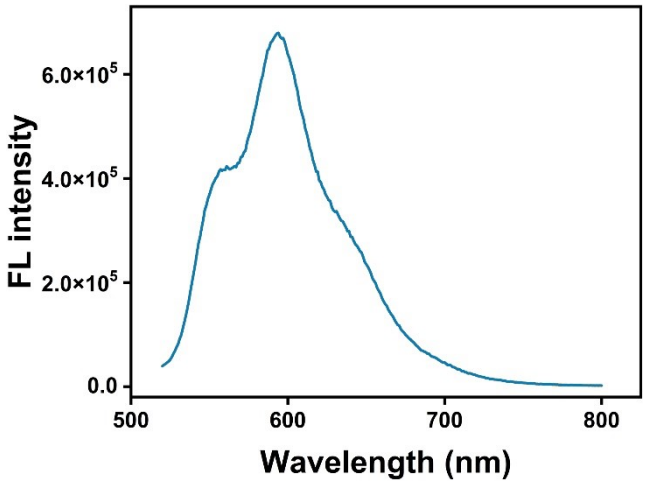

Fig. S12 Fluorescence spectrum of PDox@origami at excitation wavelength of 488 nm.

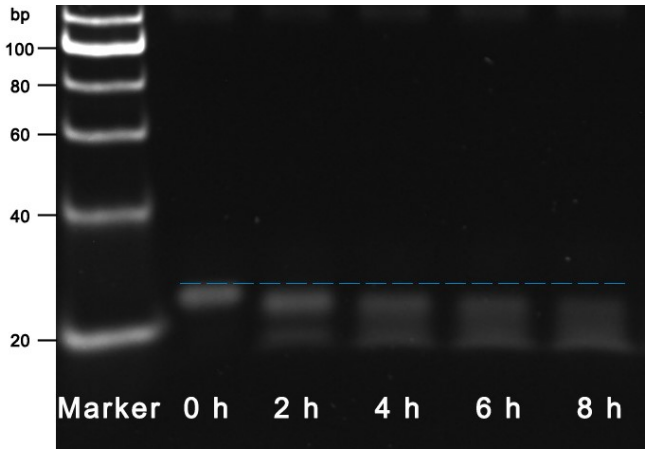

Fig. S13 PAGE gel imaging of one of the staple strands treated with 10% FBS for different time.

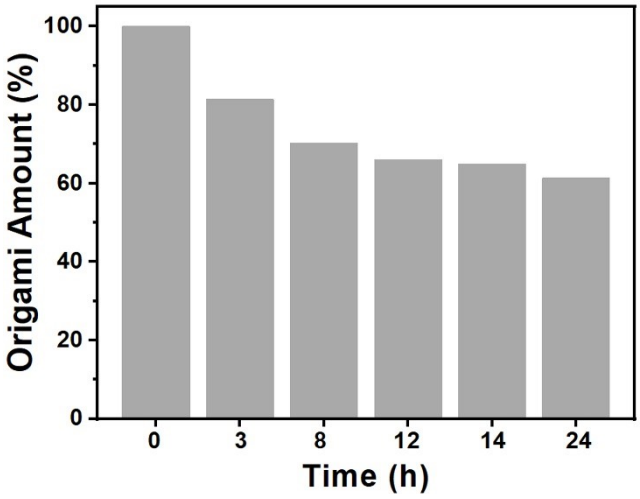

Fig. S14 Quantitative analysis of the residual rectangle DNA origami after exposure to10% FBS for various time.

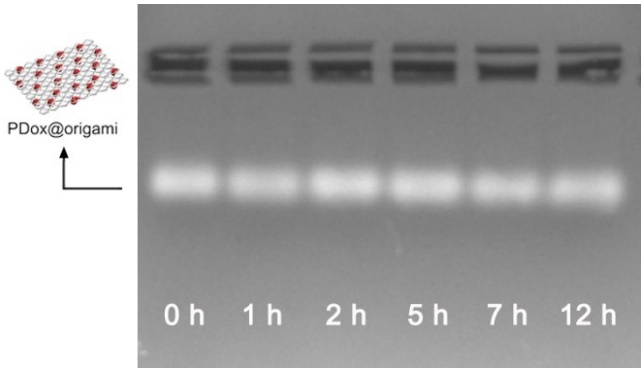

Fig. S15 Agarose gel analysis of PDox@origmai after treatment with 10% FBS for different time by recording the fluorescence of the encapsulated PDox.

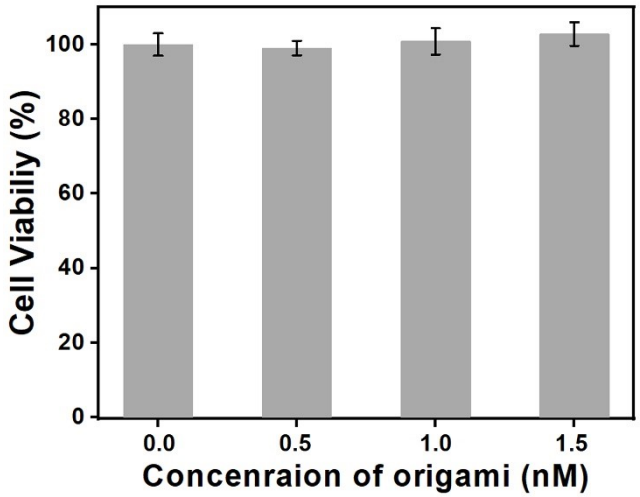

Fig. S16 Study of the biocompatibility of rectangle DNA origami. Error bars represent the standard deviation (n = 3).

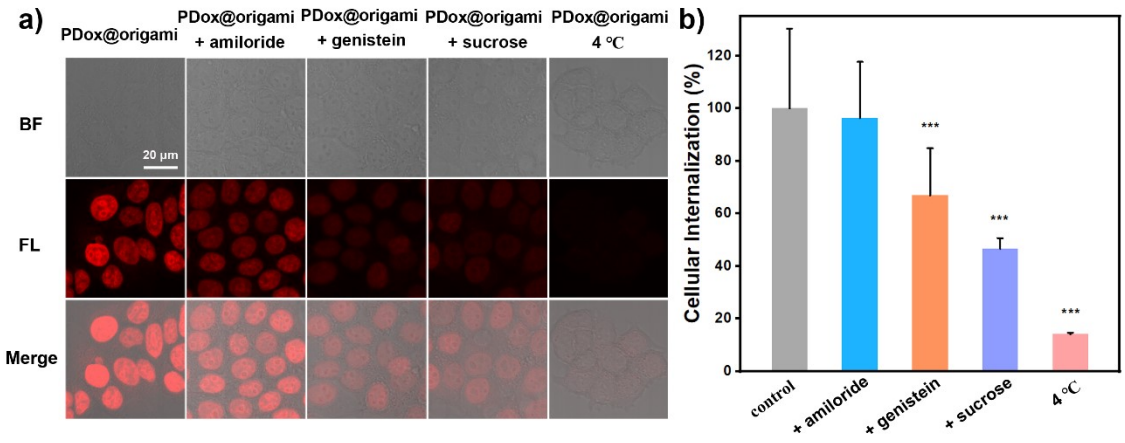

Fig. S17 Probing the endocytosis mechanism of PDox@origami by MCF-7 cells with a series of inhibitors. (a) CLSM images and (b) the corresponding quantitative analysis of intracellular fluorescence in MCF-7 cells pretreated with different inhibitors. Each quantitative analysis was recorded on at least 20 cells using Image J. ***p<0.001 *vs.* control.

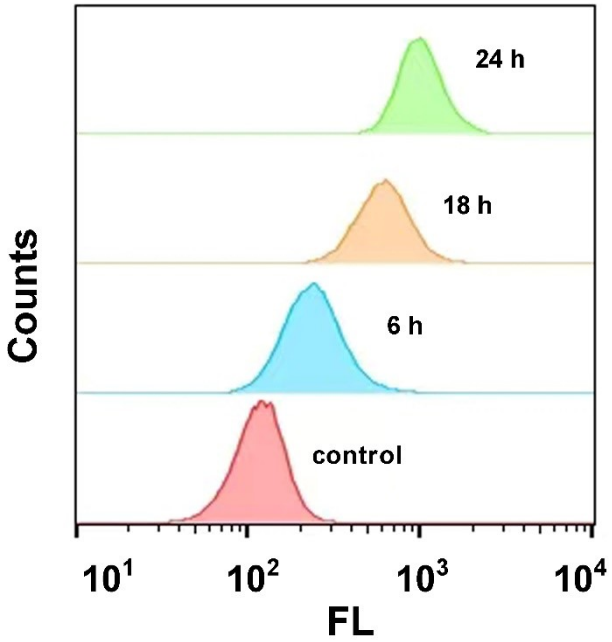

Fig. S18 Flow cytometry analysis of MCF-7 cells after incubation with PDox@origami for different periods of time (0, 6, 18, and 24 h). All experiments counted at least 10,000 cells.

Reference

1. J. Chang; W. Cai; C. Liang; Q. Tang; X. Chen; Y. Jiang; L. Mao and M. Wang, *J. Am. Chem. Soc.,* 2019, **141**, 18136–18141.
2. C. Shen; X. Lan; X. Lu; W. Ni and Q. Wang, *Chem. Commun.,* 2015, **51**, 13627–13629.