Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Endogenous stimulus-controlled estradiol@AIEgen-based covalent organic framework for reduction of myocardial ischemia/reperfusion injury

Fang Yuan,[a] Cuiling Zhang,[a] Xianzhu Luo,[a] Xiaokun Shen[b] and Yuezhong Xian*[a]*

[a]Shanghai Engineering Research Center of Molecular Therapeutics and New Drug Development, Department of Chemistry, School of Chemistry and Molecular Engineering, East China Normal University, Shanghai 200241, China

[b]Convalife (Shanghai) Co. Ltd., Shanghai 201203, China

E-mails: clzhang@chem.ecnu.edu.cn, yzxian@chem.ecnu.edu.cn.

Experimental Section

Materials and Reagent. Tetra-(4-aldehyde-(1, 1-biphenyl)) ethylene (TFBE) was purchased from Jilin Chinese Academy of Sciences-Yanshen Technology Co., Ltd. (Jilin, China). *o*-dichlorobenzene (*o*-DCB), tetrahydrofuran (THF), HCl, NaOH, were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). 1, 4-phenylenediacetonitrile (PDAN), NaCl, KCl, KH_2PO_4, $CoCl_2$, $MgCl_2$, $Na_2HPO_4 \cdot 12H_2O$ were obtained from Aladdin Industrial Co. Ltd. (Shanghai, China). ATP, CTP, GTP, TTP was purchased from Sangon Biotech Co., Ltd. (Shanghai, China). CCK-8 kit, Hoechst 33258, 3-amino, 4-aminomethyl-2', 7'-difluorescein, diacetate (DAF-FM DA) Assay Kit, Annexin V-FITC Apoptosis Detection Kit were obtained from Beyotime Institute of Biotechnology (Shanghai, China). Ultrapure water from HHitech source was applied throughout the whole experiments (Shanghai, China). DNA sequence used in this paper are as follows: (from 5' to 3')

S1-ATP:

GGGGGTTGGAAGGAGGCGTTATGATTTTACATTCCTAAGTCTGAAACATT ACAGCTTGCTACACGAGAAGAGCCGCCATAGTA

S2:

ATTTTATCACCAGGCAGTTGACAGTGTAGCAAGCTGTAATAGATGCGAGG GTCCAATAC

S3:

TCAACTGCCTGGTGATAAAACGACACTACGTGGGAATCTACTATGGCGGC TCTTC

S4:

TTCAGACTTAGGAATGTGCTTCCCACGTAGTGTCGTTTGTATTGGACCCTC GCAT

Anchor DNA: NH_2-$(CH_2)_6$-CCC AAT CGA ACC CCC

All DNA sequences were synthesized and purified from Sangon Biotech Co., Ltd. (Shanghai, China).

Characterizations. Powder X-ray diffraction (PXRD) pattern was measured by a

Smartlab SE X-ray powder diffractometer (Rigaku, Japan). Fluorescence spectra were tested on an F-7000 spectrometer (Hitachi, Japan). UV-vis absorption spectra were acquired on a UV-2250 UV-vis spectrophotometer (Shimadzu, Japan). Transmission electron microscope (TEM) was observed on a HT-7700 TEM (Hitachi, Japan). Fourier transform infrared (FTIR) spectra were performed on a Nicolet iS50 FTIR spectrometer (Thermo Fisher Scientific, USA). Hydrated size and zeta potential were acquired on a Zetasizer Nano ZS90 (Malvern, U.K.). N_2 adsorption-desorption isotherms were tested on an Autosorb-iQ gas-sorption analyzer (Quantachrome, USA). The surface areas were assessed using Brunauer-Emmett-Teller (BET) method. Cell images were obtained by a confocal laser scanning microscope (CLSM) (Nikon, Japan).

Synthesis of AIE COFs. A 10 mL Pyrex tube was added with PDAN (15.2 mg, 0.096 mmol), TFBE (35.9 mg, 0.048 mmol), NaOH aqueous solution (0.2 mL, 4 M) and 2 mL 1,4-dioxane/MeOH (V:V=1/1). Then the tube was freeze-pump-thaw for three times at 77 K (LN2 bath) and flame sealed. After recovery to room temperature, the tube was heated to 120 °C for 3 days in a vacuum oven. The obtained yellow precipitate was isolated through filtration and washed with H_2O, CH_2Cl_2, and THF sequentially. Then, the solid was Soxhlet extracted in THF for 2 days and then dried in the oven to obtain bright yellow precipitate with 55% yield.

Synthesis of AIE COFs-COOH. The AIE COFs (20.0 mg) dispersed in 20% NaOH solution (C_2H_5OH: H_2O =1:1, 10.0 mL) were charged into a round-bottomed flask. The suspension was heated to 120 °C to reflux for 3 days. Then, the solid were filtrated and washed with hot water and 1M HCl for 2 h, respectively. After that, the precipitate was collected by filtration, and washed with H_2O and THF. The precipitate was then Soxhlet extracted with THF and dried under vacuum at 80 °C to obtain AIE COFs-COOH as a yellow powder (16.1 mg, 80.5%).

ssDNA modification of AIE COFs (ssDNA/COFs). The AIE COFs-COOH (0.5 mg) was suspended in 1.0 mL, phosphate buffered saline solution (PBS, 10.0 mM, pH = 7.4). Then, 2.5 mg of EDC and 2.5 mg of NHS were added and stirred under dark place to activate carboxyl for 30 min. After that, 25 μL (100 nmol) of ssDNA was added and shaken gently overnight. Finally, the ssDNA/COFs were obtained by centrifugation and

washed with PBS for 3 times to remove unreacted ssDNA.

tDNA self-assembly. The ATP aptamer-modified tDNA were fabricated as follows: 2 μM of (S1-ATP), (S2), (S3), (S4) dissolved in 10.0 mM HEPES buffer (20.0 mM $MgCl_2$, pH = 7.2) were mixed and annealed at 95 ^oC for 5 min and cooled down to 25 ^oC for 2 h to yield the tDNA.

E2 loading. ssDNA/COFs (0.5 mg) were suspended in 2.4 mL E2 solutions (1.0 mg in PBS/C_2H_5OH (v/v=1:5) mixture) and stirred for 24 h at room temperature. Then, E2-loaded ssDNA/COFs were centrifuged, washed with ethanol and dried at room temperature. Subsequently, the as-obtained E2-loaded ssDNA/COFs were re-suspended in 1.0 mL PBS (10.0 mM, pH=7.4), followed by addition of 100 nM tDNA (90 μL) and reaction for 2 h under shaking conditions. After that, the E2-tDNA/COFs were obtained by centrifuged and washed with PBS for 3 times to remove the excess tDNA. The amount of E2 loaded in COFs was measured based on the fluorescence intensity of E2 with excitation wavelength at 284 nm and emission wavelength at 315 nm. The drug loading efficiency and loading capacity were calculated by the following equations,

$$Loading\ efficiency\ (LE)\ \% = \frac{weight\ of\ E2\ in\ COFs}{weight\ of\ E2\ fed\ initially} \times 100\%$$

$$Loading\ capacity\ (LC)\ \% = \frac{weight\ of\ E2\ in\ COFs}{weight\ of\ E2\ in\ COFs + weight\ of\ COFs} \times 100\%$$

ATP-Responsive E2 releasing from E2-tDNA/COFs. The E2-tDNA/COFs (1.0 mL) suspension was pipetted into a dialysis membrane ($MW_{cut\ off}$ = 8-14 kDa) and then introduced 15.0 mL PBS solutions (10.0 mM, pH 7.4) containing 0.5% (v/v) Tween 80 with or without 10.0 mM ATP. The solutions were stirred at room temperature. After different incubation time, 0.2 mL of the medium was withdrawn for E2 measurement and 0.2 mL of fresh buffer was simultaneously added. E2 release was determined by a fluorescence spectrophotometer according to the calibration curve. The E2 release rates at different time (t) were calculated by the following equation,

$$E2\ release\ rate = \frac{the\ cumulative\ release\ amount\ of\ E2\ at\ time\ t}{the\ total\ amount\ of\ E2\ loaded\ in\ COFs} \times 100\%$$

For the selective release experiments, an aqueous solution of the E2-tDNA/COFs (1.0

mL) was pipetted into a dialysis membrane and introduced into 15.0 mL PBS solutions (10.0 mM, pH 7.4) containing 0.5% (v/v) Tween 80 with 10.0 mM ATP, GTP, CTP or TTP, respectively. The solutions were continuously stirred at room temperature. After 9 h, 0.2 mL of the medium was taken and E2 release was tested using a fluorescence spectrophotometer.

Cell culture. H9C2 rat heart myoblasts were purchase from Stem Cell Bank, Chinese Academy of Sciences (Shanghai, P. R. China) and cultured in Dulbecco's minimal essential medium (DMEM), containing of 10% fetal bovine serum (FBS), 1% penicillin and streptomycin. They were incubated in a humidified, 5.0 % CO_2 incubator. The cells were cultured in 25 cm^2 cell culture flasks and the culture medium was refreshed every 2 days. The cells were passaged or seeded using 0.25% trypsin-EDTA when the cell's confluency was about ~80% every 2-4 days.

The cytotoxicity of tDNA/COFs. The H9C2 cells were seeded in a 96-well plates (1.0×10^5 cells per well) for 12 h. The cells were treated with different concentration of tDNA/COFs (0, 10, 25, 50, 100, 200 μg mL^{-1}) for 24 h. Then, the cells were washed with PBS for 3 times and added with 100 μL of DMEM solutions each well containing 10 μL CCK-8 solution for another 4 h incubation. The absorbance at 450 nm was tested using a microplate reader.

Cell uptake of tDNA/COFs. The H9C2 cells were seeded into a 24-well plate and incubated overnight. Then, the culture medium was renewed with fresh DMEM containing 50 μg mL^{-1} tDNA/COFs and incubated for different time (1, 2, 4, 6 h). At each time point, the cells were washes with PBS carefully and fixed with 4% polyformaldehyde, stained with Hoechst. Finally, the cells were subjected to CLSM for imaging. (Excitation: 405 nm; emission: 470 ± 25 nm for blue channel, and excitation: 488 nm; emission: 500 ± 25 nm for green channel).

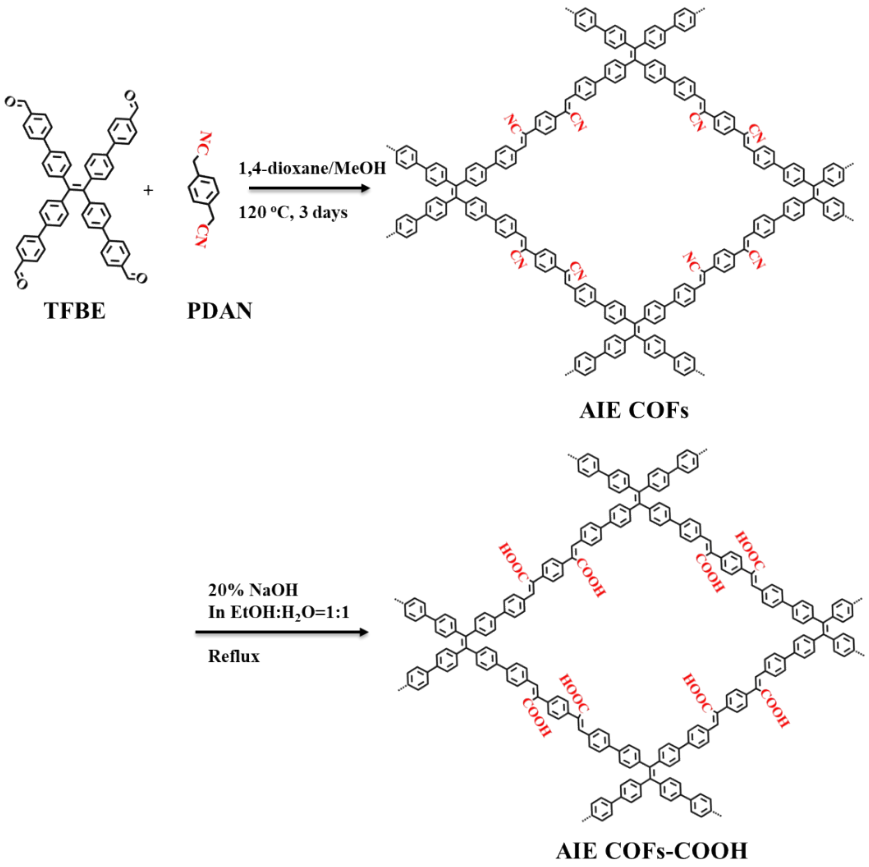
Cytoprotective effects of E2-tDNA/COFs in H9C2 cell under I/R model. The H9C2 cells were seeded into a 96-well plate with fresh DMEM for growth overnight. Then, the cells washed with PBS for 3 times and incubated in 500.0 μM $CoCl_2$ solution for 4 h to induce ischemia. After that, the cells were reperfusion in fresh DMEM containing different concentration of E2-tDNA/COFs or free E2 for 4 h. Next, the incubation

media was discarded and the cells were washed with PBS for 3 times. Finally, the cell viabilities were assessed by CCK-8 assays.

Visualization of intracellular NO. DAF-FM DA was selected as a NO specific fluorescence probe to visualize intracellular NO. The H9C2 cells were seeded into a 24-well plate with a density of 1.0×10^4 cells in fresh DMEM for growth overnight. Then the cells were stained with DAF-FM DA (5.0 μM) for 30 min and subjected to 500.0 μM $CoCl_2$ solution for 4 h and then reperfusion in DMEM containing free E2 (0.142 nM) or E2-tDNA/COFs (50 μg mL^{-1}) for 4 h. After that, the cells were washed with PBS, fixed with 4% polyformaldehyde, and subjected to CLSM imaging under excited at 488 nm, and collected at 470 ± 50 nm.

Cell metabolism of E2-tDNA/COFs. The H9C2 cells were seeded into a 24-well plate and incubated overnight. Then, the cells were washed with PBS and incubated in 500.0 μM $CoCl_2$ solution for 4 h to induce ischemia. After that, the cells were reperfusion in fresh DMEM containing 50 μg mL^{-1} E2-tDNA/COFs for 4 h. Next, the cells were washes with PBS and cultured in fresh culture medium for another 12, 24, 48 and 72 h, respectively. Finally, the cells were fixed with 4% polyformaldehyde, stained with Hoechst and subjected to CLSM imaging. (Excitation: 405 nm; emission: 470 ± 25 nm for blue channel, and excitation: 488 nm; emission: 500 ± 25 nm for green channel).

Flow Cytometry. The H9C2 cells were seeded into a 6-well plate with fresh DMEM for growth overnight. Then, the cells were washed with PBS for 3 times and incubated in 500.0 μM $CoCl_2$ solution for 4 h to induce ischemia. After that, the cells were reperfusion in fresh DMEM containing free E2 (0.142 nM) or E2-tDNA/COFs (50 μg mL^{-1}) for 4 h. Next, the cells was digested and stained with Annexin V-FITC for 30 min, then the samples were analyzed by flow cytometry.



Scheme S1 The synthesis and post-modification of AIE COFs.

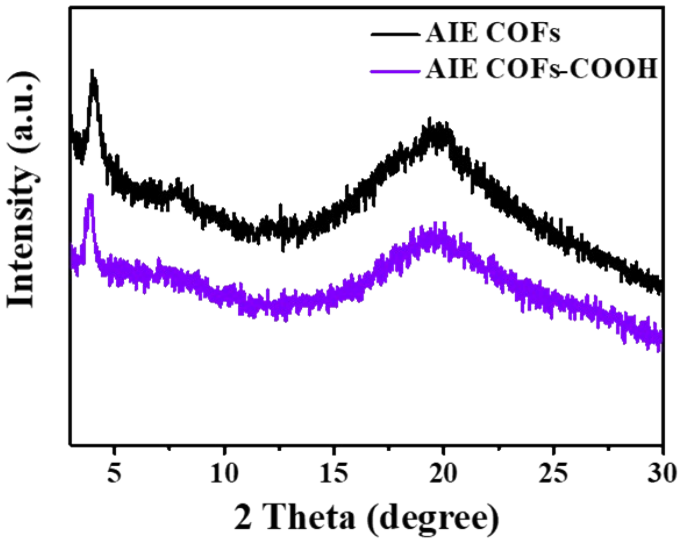


Fig. S1 XRD patterns of AIE COFs and AIE COFs-COOH.

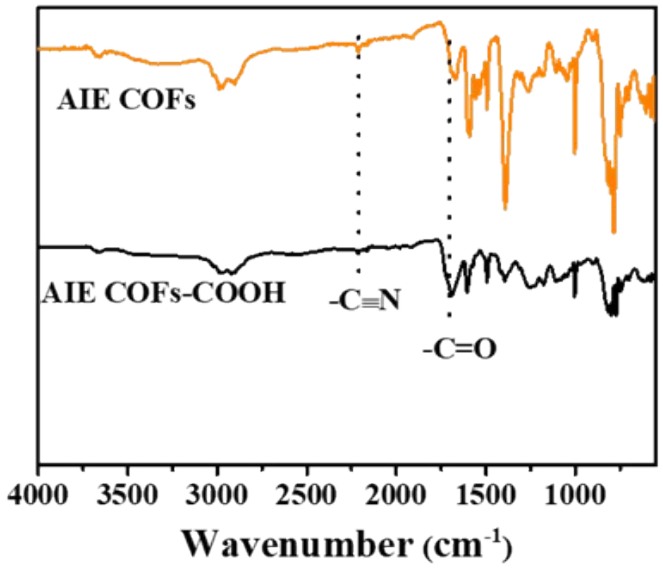


Fig. S2 FITR spectra of AIE COFs and AIE COFs-COOH.

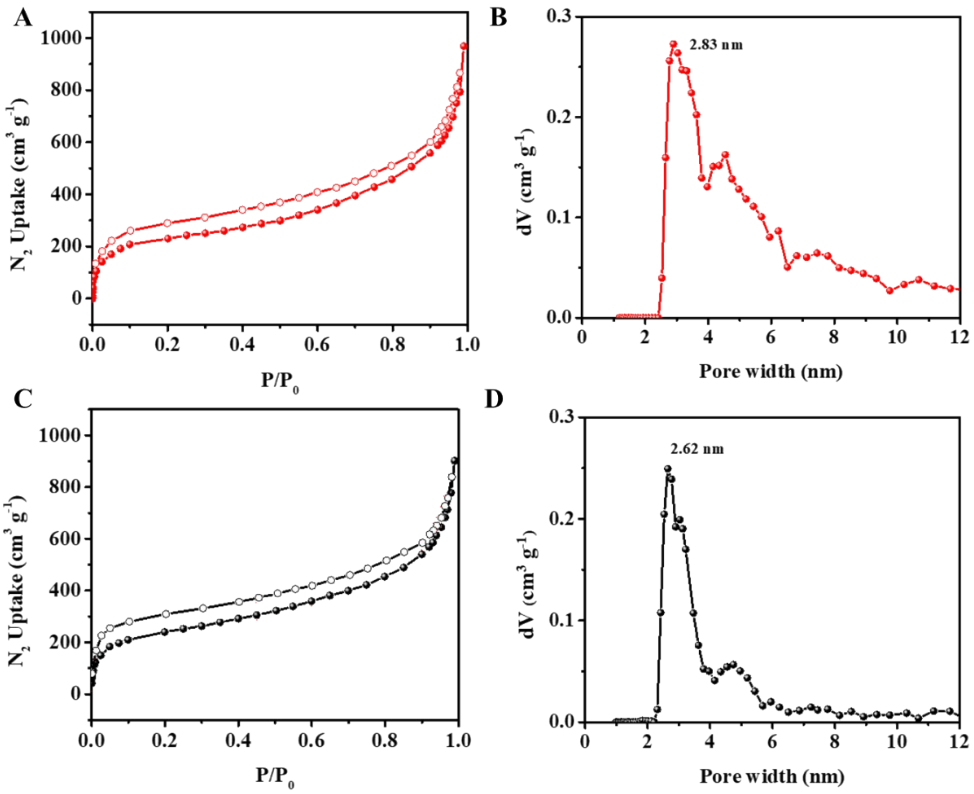


Fig. S3. N_2 adsorption (filled symbols) and desorption (empty symbols) isotherms of (A) AIE COFs and (B) AIE COFs-COOH. Pore size distribution curves of (C) AIE COFs and (D) AIE COFs-COOH.

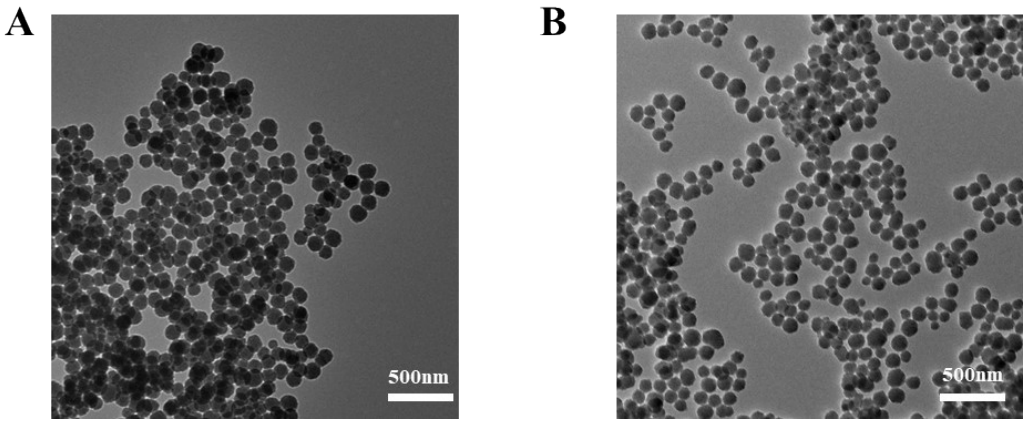


Fig. S4 TEM images of (A) AIE COFs and (B) AIE COFs-COOH.

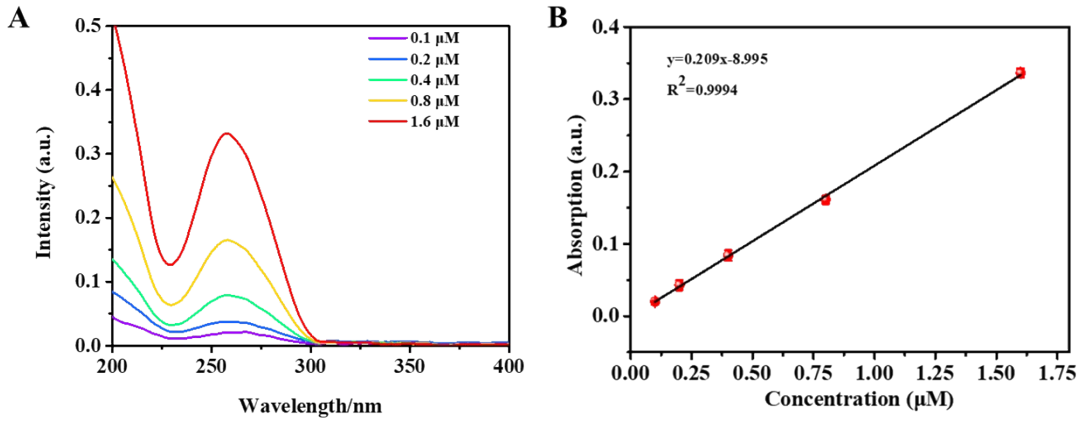


Fig. S5 (A) UV-Vis absorption spectra of different concentrations of ssDNA. (B) Linear relationship between the UV-Vis absorption at 260 nm and the concentration of ssDNA.

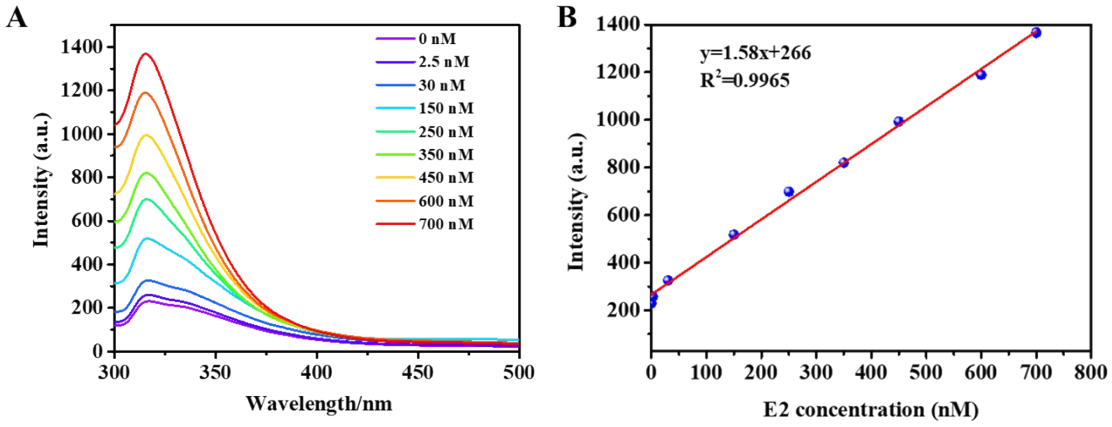


Fig. S6 (A) Fluorescence spectra of different concentrations of E2. (B) Linear relationship between the fluorescence intensity and the concentration of E2.

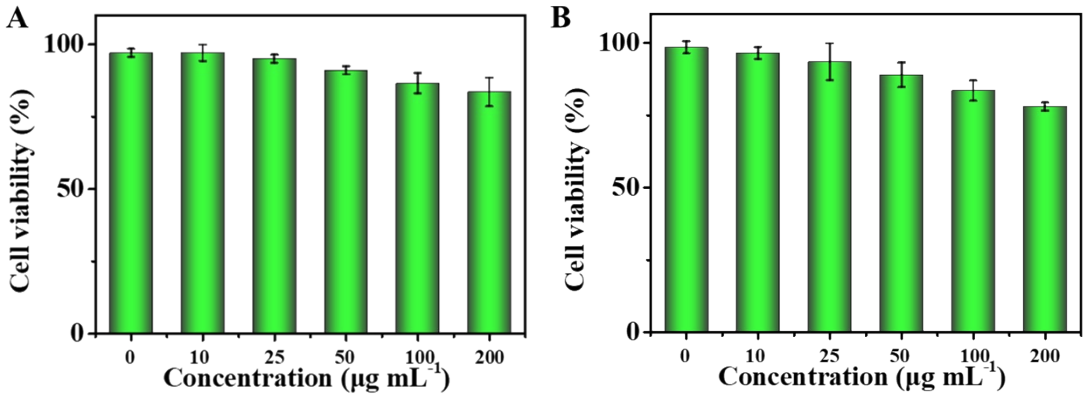


Fig. S7 Cell viability of H9C2 cells after treated with different concentrations of tDNA/COFs (A) at 24 h, (B) at 48 h.

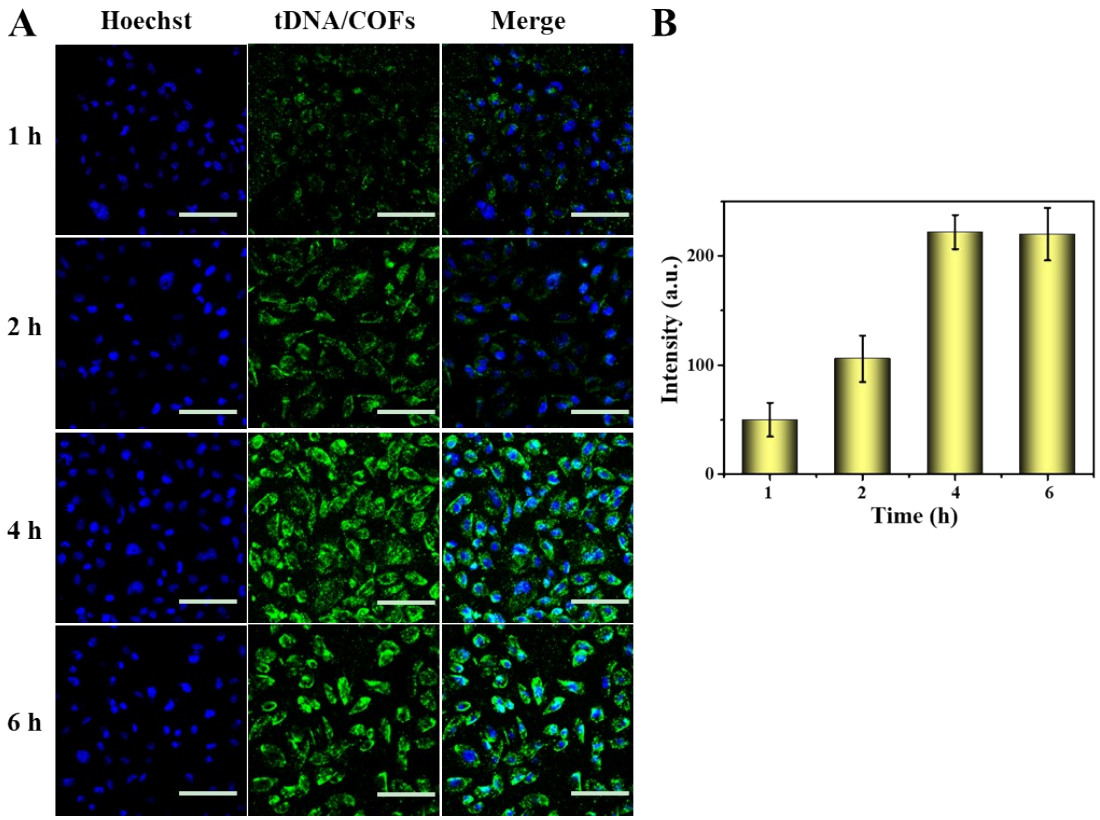


Fig. S8 (A) CLSM images of H9C2 cells treated with tDNA/COFs for different time. Scale bar = 100 μm. (B) Corresponding mean fluorescence intensity of H9C2 cells in CLSM images.

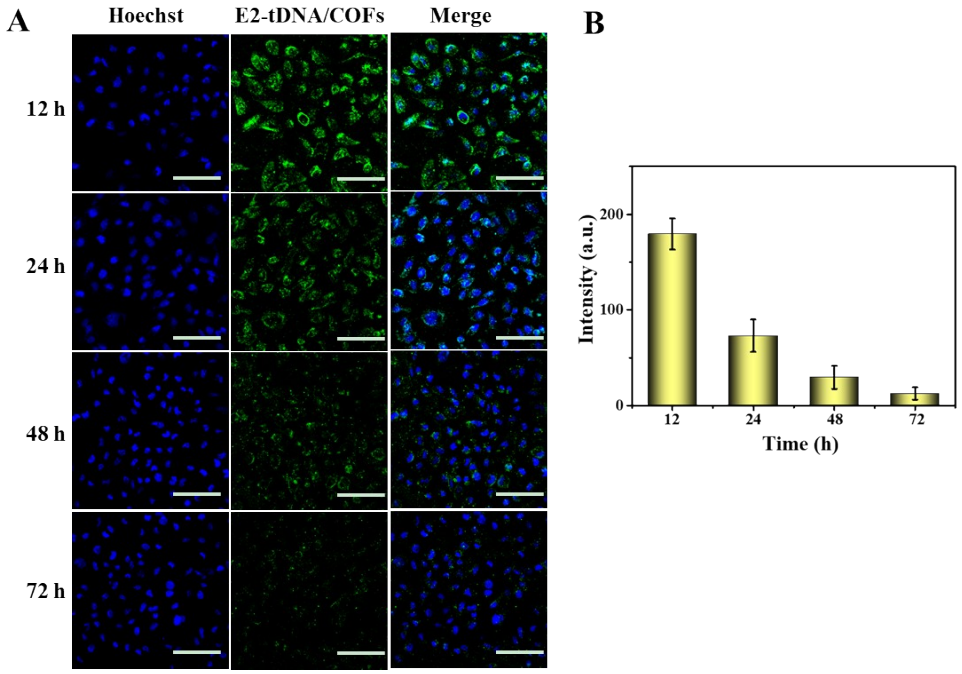


Fig. S9 CLSM images of E2-tDNA/COFs treated H9C2 cells under different metabolism times. Scale bar = 100 μm.