Electronic Supplementary Material (ESI) for Materials Chemistry Frontiers.

Supporting Information

Photoactive conjugated polymer/graphdiyne nanocatalyst for CO_2 reduce into CO in living cells for hypoxia tumor treatment

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Experimental Section

Materials.

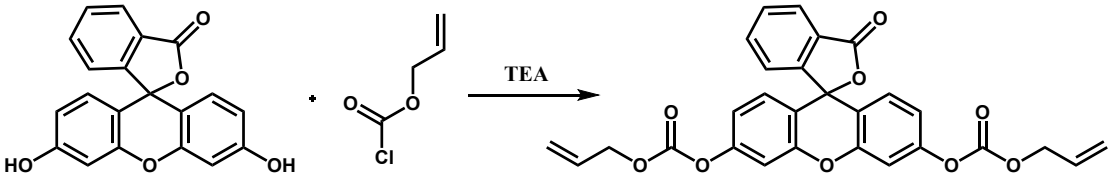
Cationic PBF and PFP were synthesized according to the reported literatures [1-2]. Graphdiyne (GDY) was present by Prof. Yuliang Li group from Institute of Chemistry, Chinese Academy of Sciences. All chemical reagents were commercially available and used without further purification. 4T1 cells were purchased from cell culture center of the Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences. Roswell Park Memorial Institute 1640 medium (RPMI 1640), Dulbecco Modified Eagle Medium (DMEM), penicillin-streptomycin and phosphate buffer saline (PBS) were purchased from Hyclone (Beijing, China). Fetal bovine serum (FBS) was acquired from Sijiqing Biological Engineering Materials (Hangzhou, China). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was commercially obtained from Xinjingke Biotechnology Co., Ltd (Beijing, China). Mitochondrial membrane potential assay kit (JC-1), ATP and BCA Assay Kits were provided by Beyotime Biotechnology. Deionized water was obtained from a Milli-Q system (Millipore, Bedford, MA). Hoechst 33342, Lyso Tracker™ Green DND and Mito Tracker™ Green FM were provided by Life Technologies.

Instruments.

UV-Vis absorption spectra were taken on a Thermo Scientific Evolution 201 spectrophotometer and the fluorescence spectra were measured using a Hitachi F-4500 fluorometer equipped with a Xenon lamp excitation source. Gas chromatography measurements wer profrmed on Agilent 8860 system with MolSieve 5A 60/80 ss 8Ft 1/8 2mm. Zeta potential and dynamic dynamic light scattering measurements were performed on Malvern Zetasizer Nano ZS 90. Transmission electron microscopy images were taken on HT7700 microscope (100 kV, Japan). Calorimetric measurements were taken on a TAM 2277-201 microcalorimetric

system (Thermometric AB, Järfälla, Sweden). X-ray photo-electron spectra were reconrded on the spectroscopy (Kratos Axis Ultra DLD, England). Electrochemitry measurements were conducted on an electrochemical workstation (Metrohm, Autolab PGATAT 302N, Switzerland). The MTT assay were recorded on a microplate reader (BioTek Synergy HT, USA). Confocal laser scanning microscopy (CLSM) images were conducted on confocal laser scanning biological microscope (Olympus FV 1200-BX61, Japan).

Synthesis of C,C'-(3-oxo[irp[isobenzofuran-1(3H),9'-[9H]xanthene]-3',6'-diyl]C,C'-di-propen-1-yl ester (Fl-CO).



FI-CO was synthesized according to the literature [3]. Under Ar atmosphere, fluorescein (664 mg, 2 mmol) was dissolved in super dry THF followed by drowsily adding TEA (850 μL), the mixture was stirred for 30 min at room temperature. Then, allyl chloroformate (486.6 μL, 8 mmol) was slowly added into the above reaction system within 10 min under ice bath, and stirred overnight. After the reaction, the solution was filtered and washed with brine and deionized water for three times separately, the organic solution was concentrated in vacuum after dried over $MgSO_4$. The residue was purified by silica gel chromatography with ethyl acetate/hexane (v/v=1/2) to afford compound **2** (4.9 g, 73%) as yellow solid. ^1H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.05 (d, J=7.2, 1H), 7.71 (dd, J_1=1.1, J_2=7.3 2H), 7.20 (d, J=2.2, 2H), 6.93 (d, J=1.4, 2H), 6.90 (d, J=2.3, 2H), 6.05 (ddt, 2H), 5.46 (m, 4H), 4.76 (d, J=5.9, 4H). HR-MS (ESI): calcd for $[C_{28}H_{21}O_9]^+$ ($[M]^+$) 501.118009, found 500.118156.

Preparation of CPs@GDY/DSPE-PEG nanocatalyst.

Briefly, 3 mg GDY was dispersed in 1 mL DI water and treat with ultrasound for 4 h, then

0.75 mL conjugated polymer (PFP or PBF, 4 mg/mL) was added and treat with ultrasound for another 1 h. CPs@GDY solution was further washed with DI water by centrifugal filter units (100 kDa, Millipore). Then obtained soluton was added in to 15 mL DSPE-PEG2000 (20 mg/mL) soluiton and treated with ultrasound for 4 h. CPs@GDY/DSPE-PEG nanocatalys was further washed and concentrated with DI water by centrifugal filter units (100 kDa, Millipore). The obtained stock solution was kept in the dark at 4 ℃.

Gas chromatography measurements.

GDY, PFP@GDY/DSPE-PEG and PBF@GDY/DSPE-PEG was dilluted at concentration of 20 μg/mL in 2 mL $NaHCO_3$ buffer (pH 8.5), respectively. After bubbling the dilluted solution with N_2 for 10 min, the pure CO_2 or mimetic hypoxia atmosphere (1% O_2, 5% CO_2 and 94% N_2) was pumped into a 4 mL transparent glass bottle to exchange the top air. Following by a light irradiation of 80 mW/cm^2 for 4 h, CO yield was determined by gas chromatography. In order to get CO standard curve, 0.5% and 1% CO was detected through GC, respectively.

DLS and Zeta potential measurements.

5 μg/mL GDY in water was added to the cuvette then the size was measured. Mixture of 5 μg/mL GDY and 5 μg/mL CPs was incubated at 37 °C for 30 min, and the complex solution was used to measure the size. Zeta potential measurements were also conducted by the protocols above.

TEM image.

Stock solution of CPs@GDY/DSPE-PEG (500 μg/mL) was diluted to 20 μg/mL in water. 5 μL of the solution was added on ultra-thin carbon network. After the solvent was lyophilized, the samples were observed using TEM.

Photocurrent characterizations.

The mixture of 30 μL GDY (40 μg/mL), 30 μL CPs (40 μg/mL) and 60 μL GDY (40 μg/mL) and 60 μl CPs (40 μg/mL) were dripped onto 1 cm × 1 cm carbon paper respectively. 20 μl 0.05% Nafion aqueous solution was added carbon paper electrode after the solvent volatilizing. Using carbon paper with treatment as the working electrode, Ag/AgCl as reference electrode and Pt electrode as counter electrode, the photocurrent was measured by CHI1040 under light intensity of 50 mW/cm^2. The applied voltage was 0.2 V and the scanning rate was 0.05 mV/s.

Isothermal Titration Microcalorimetry (ITC) measurements.

Briefly, the cell was loaded with 0.6 mL of GYD solution. The PBF or PFP solution was injected into the cell by a 500 μL Hamilton syringe controlled by a 612 thermometric lund pump. The system was stirred at 60 rpm with a gold propeller. All of the measurement was performed at 25.00 ± 0.01 ℃. The accuracy of the calorimeter was periodically calibrated electrically and verified by measuring the dilution enthalpy of concentrated sucrose solution. The experiments were repeated at least twice with deviation within ± 4%.

Measurement of ROS production.

Briefly, 5 μL PBF (500 μg/mL) or PFP(500 μg/mL) or PBF@GDY/DSPE-PEG (500 μg/mL) or PFP@GDY/DSPE-PEG (500 μg/mL) solution was added into a well of 96-well plate, then 10 μL sodium ascorbate (200 mM, to remove oxygen) and 185 μL DCFH solution was added, respevtivley. The palte was irradiated with white light (40 mW cm^{-2}), and the fluorescence intensity of the solution at 525 nm was recorded every minute with the excitation wavelength of 488 nm.

Cell Culture.

4T1 cells were cultured in RPMI 1640 supplemented with 10% FBS in a humidified incubator containing 5% CO_2 at 37 °C.

Cell viability assay by MTT.

Briefly, 4T1 cells were seeded in 96-well plates at a density of 8×10^3 cells/well and cultured in medium at 37 ℃ for 12 h. Then, the medium containing different concentrations of CPs@GDY/DSPE-PEG nanocatalyst and TEOA was utilized to treat 4T1 cells at 37 ℃ for 24 h. The culture medium was discarded and MTT (0.5 mg/mL in PBS, 100 μL/well) was added to the wells followed by incubation at 37 °C for another 4 h. The supernatant was removed again and DMSO (100 μL/well) was added to dissolve the produced formazan. After shaking the plates for 3 min, all the absorbance of the wells was read with a microplate reader at 570 nm. The cell viability rate (VR) was calculated according to the following equation:

$$VR = A/A_0 \times 100\%$$

where A was the absorbance of the experimental group and A_0 was the absorbance of the control group.

Cell Co-localization analysis of CPs@GDY/DSPE-PEG nanocatalyst.

Briefly, 4T1 cells were seeded in confocal dishes at a density of 5×10^4 cells/well and cultured in medium at 37 ℃ for 24 h. The medium was removed and the cells were cultured in medium containing CPs@GDY/DSPE-PEG nanocatalyst (5 μg/mL) at 37 ℃ overnight. The medium was removed again and the cells were washed with PBS for three times. Various dyes (1 μM in medium) including Hoechst 33342, Lyso Tracker™ Green DND and 1 μM Mito Tracker™ Green FM were cocultured with cells for 30 min separately. The CLSM images were taken by confocal laser scanning microscopy. The fluorescence imaging was collected at 500-530 nm (λex: 488 nm), 590-600 nm (λex: 488 nm), respectively.

Intracellular CO production by CPs@GDY/DSPE-PEG nanocatalyst.

Briefly, 4T1 cells were seeded in confocal dishes at a density of 5×10^4 cells/well and cultured in medium at 37 ℃ for 24 h. The cells were cultured in medium containing CPs@GDY/DSPE-PEG nanocatalyst (5 μg/mL) and TEOA (1 mM) at 37 ℃ overnight. The medium was removed again and the cells were cultured in medium with TEOA (1 mM) at 37 ℃ for another 6 h. Then, the cells were irradiated with white light (40 mW cm^{-2}) for 20 min, and subsequently incubated with Fl-CO (1 μM) and $PdCl_2$ (1 μM) for 30 min. The CLSM images were taken by confocal laser scanning microscopy. The fluorescence imaging was collected at 500-530 nm (λex: 488 nm), 590-600 nm (λex: 488 nm), respectively.

Mitochondrial membrane potential measurement.

Briefly, 4T1 were seeded in confocal dishes at a density of 5×10^4 cells/well and cultured in medium at 37 ℃ for 24 h. The cells were cultured in medium containing CPs@GDY/DSPE-PEG nanocatalyst (5 μg/mL) and TEOA (1 mM) at 37 ℃ overnight. The medium was removed again and the cells were cultured in medium with TEOA (1 mM) at 37 ℃ for another 6 h. Then, the cells were irradiated with white light (40 mW cm^{-2}) for 20 min, and subsequently incubated with JC-1 (5 ×) for 30 min. Then cells were washed with JC-1 (1.25 ×) for three times. The CLSM images were taken by confocal laser scanning microscopy. The fluorescence imaging was collected at 500-530 nm (λex: 488 nm), 590-600 nm (λex: 488 nm), respectively.

Phototoxicity of nanocatalyst and CPs by MTT assay in hypoxia condition.

Briefly, 4T1 cells were seeded in 96-well plates at a density of 8×10^3 cells/well and cultured in medium at 37 ℃ for 12 h. Then, the medium containing different concentrations of CPs@GDY/DSPE-PEG nanocatalyst was utilized to treat 4T1 cells at 37 ℃ for 24 h. Then the cells was incubated in 1% O_2, 5% CO_2 at 37 ℃ for 6 h. After irradiation of 50 mW/cm^2 for 20

min, the cells were incubated in 5% CO_2 at 37 ℃ for 40 h. The culture medium was discarded and MTT (0.5 mg/mL in PBS, 100 μL/well) was added to the wells followed by incubation at 37 °C for another 4 h. The supernatant was removed again and DMSO (100 μL/well) was added to dissolve the produced formazan. After shaking the plates for 3 min, all the absorbance of the wells was read with a microplate reader at 570 nm. The cell viability rate (VR) was calculated according to the following equation:

$$VR = A/A_0 \times 100\%$$

where A was the absorbance of the experimental group and A0 was the absorbance of the control group.

ATP level assay.

4T1 cells were seeded in 6-well plates at a density of 1×10^5 cells/well and cultured in medium at 37 ℃ for 12 h. Then, the medium containing different concentrations of 5 μg/mL CPs@GDY/DSPE-PEG nanocatalyst and 1 mM TEOA was utilized to treat 4T1 cells at 37 ℃ for 12 h. The culture medium was discarded and 1 mM TEOA (cell medium) was added to the wells followed by incubation at 1% O_2, 5% CO_2 37 °C for another 6 h. After irradiation of 50 mW/cm^2 for 20 min, the cells were addition of cell lysis buffer. Then intracellular ATP level was measured by ATP kit.

References

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3. Feng WY, Liu DD, Feng SM, Feng GQ, Anal Chem, 2016, 88: 10648−10653

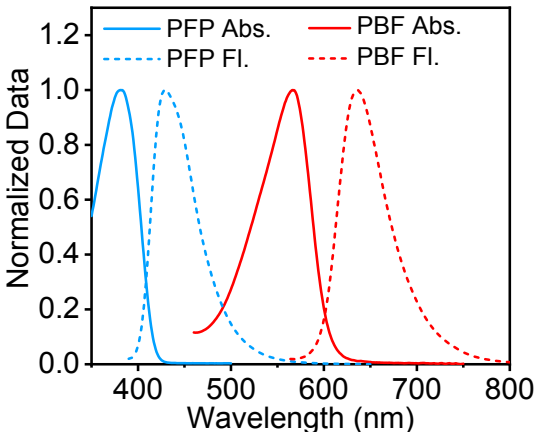

Figure S1. Absorption and fluorescence spectra of PFP and PBF.

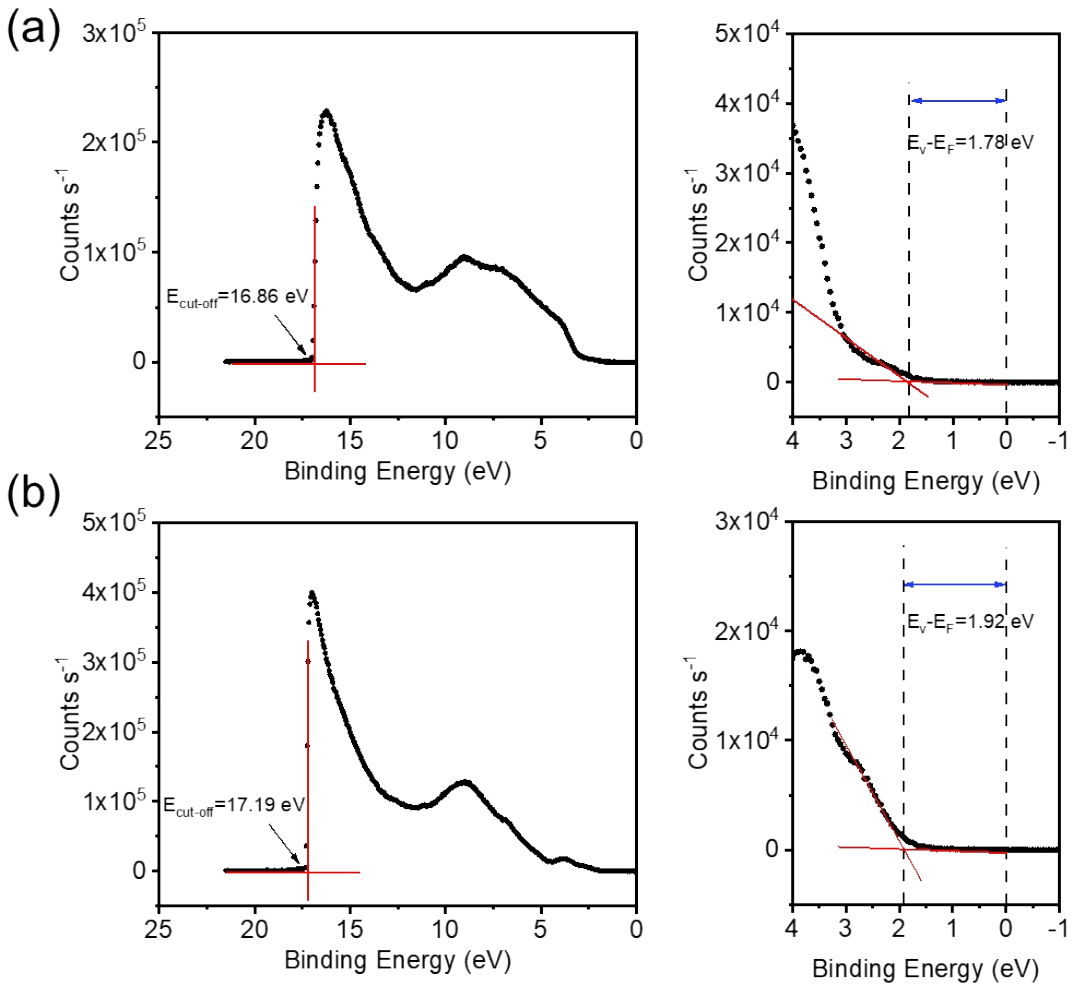

Figure S2. UPS analysis of conjugated polymer PFP (a) and PBF (b).

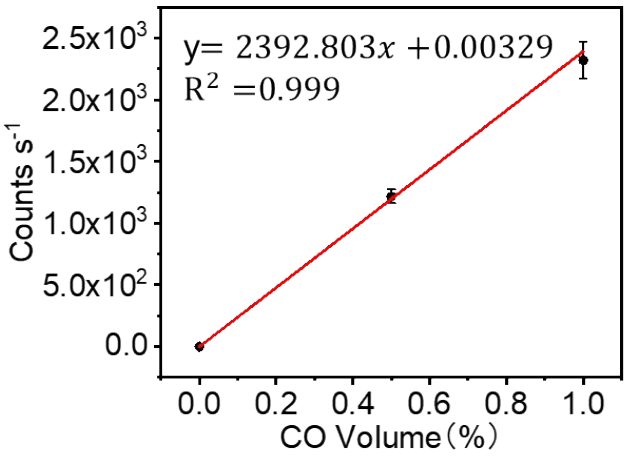

Figure S3. Standard curve of CO was tested by GC.

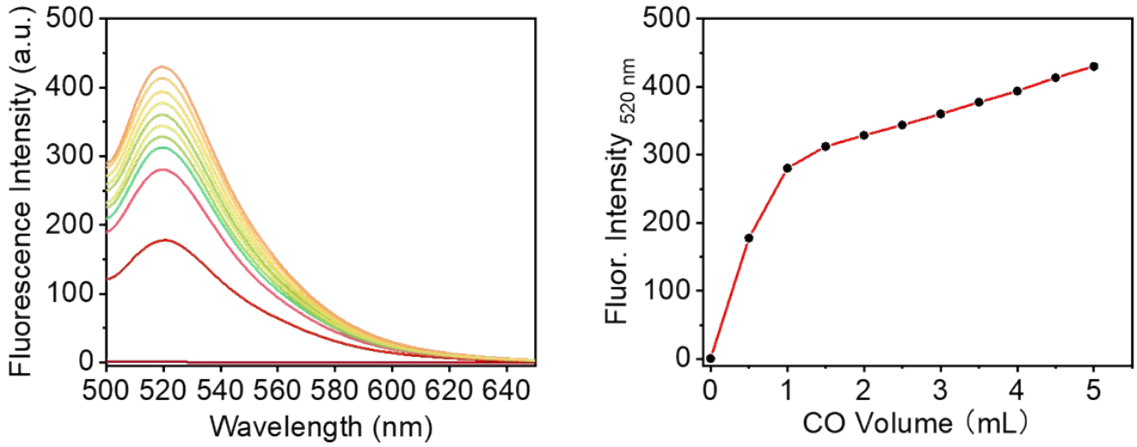

Figure S4. The fluorescence of FL-CO towards different concentration CO and plot of fluorescence at 520 nm.

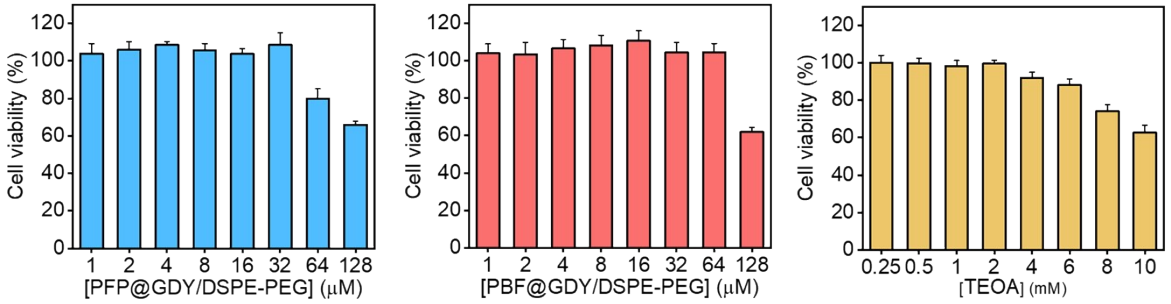

Figure S5. 4T1 cell viability with treatment of PBF@GDY/DSPE-PEG, PFP@GDY/DSPE-PEG and TEOA by MTT assay.