Electronic Supplementary Information

Morpholine-Modified Permethyl β-Cyclodextrin Supramolecular Nanoparticles for Precise Dual-Targeted Imaging

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1. General information

Materials. All reagents and solvents were obtained from commercial suppliers and used as received unless specified otherwise. All aqueous solutions were prepared with distilled water. 6-deoxy-6-azido-permethyl-β-CD (N_3-PCD),[S1] was prepared according to the reported procedures. Chitosan quaternary ammonium salt was purchased from Shanghai Yuanye Biotech, and the molecular weight is 100,000 with 90% substitution of quaternary ammonium.

Purification and characterization. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DMX 400 MHz spectrometer. High-resolution mass spectrum was recorded on Varian 7.0 T FTMS with the MALDI ion source. Fourier Transform Infrared spectra (FT-IR) were recorded on Bruker TENSOR II. TEM images were obtained on a Tecnai G2F20 microscope (FEI) at an accelerating voltage of 200 kV. The samples were prepared by placing a drop of solution onto a carbon-coated copper grid and air-drying it. UV-Vis spectra were recorded in a quartz cell (light path = 1 cm) on a Shimadzu UV-3600 spectrophotometer equipped with a PTC-348WI temperature controller. Zeta potentials and DLSs were measured by Brookhaven instrument. The fluorescent confocal images were carried out on an Olympus FV1000 fluorescence microscope. The white light irradiation experiment was carried out by employing a CEL-HXUV300 xenon lamp with a >420 nm cut-off filter at a power density of 220 mW/cm^2.

Cell culture. Human cervical cancer Hela cell line and human embryonic lung fibroblast MCR-5 cell line were obtained from Institute of Basic Medical Science, Chinese Academy of Medical Science. Hela and MRC-5 cells were cultured in a cell incubator with a DMEM high-glucose nutrient medium containing 10% fetal bovine serum and 1 % penicillin streptomycin in a humidified standard under 5% CO_2 at 37 °C.

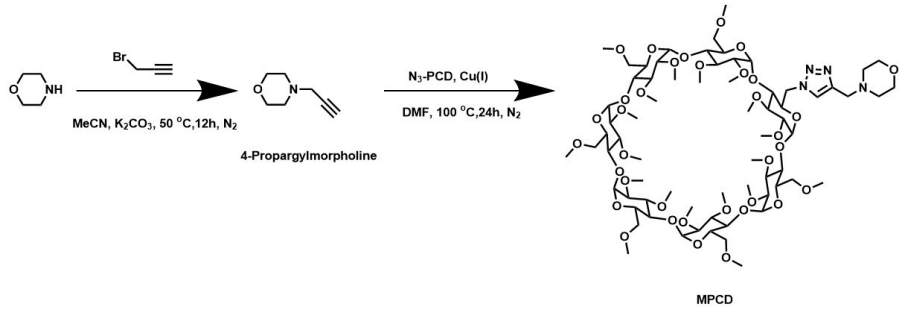
Cell uptake and colocalization imaging: The Hela cells were first subcultured into a confocal petri dish and incubated for 24 h. Then cells were treated with TPPS, TPPS⊂MPCD, FACA/TPPS⊂MPCD solution that final concentration is 10 μM ([TPPS]) in the culture medium and cultured for another 24 h. After this, the culture medium was discarded, and the cells were washed with 0.01 M PBS at least three times. Next, LysoTracker Green cocultured with the cells at 37 °C for 30 min to stain the lysosomes. After the cells were repeatedly washed at least three times with PBS, the localization of the nanoparticles in the cells was immediately observed by CLSM.

CCK8 assay: The cells were seeded in 96-well plates at a density of 5 × 10^4 cells per well in 100 μL culture medium and cultured in 5% CO_2 at 37 °C for 12 h. Then the cells were incubated with TPPS, TPPS⊂MPCD complex, FACA/TPPS⊂MPCD assembly, and further incubated for 24 h, respectively. After being irradiated under the white light for 10 min, the cells were washed and replenished with fresh culture medium, and further incubated for 1 h. The cell viability was evaluated by CCK8 assay according to the kit instruction. The plate was then read by a microplate reader at a wavelength of 450 nm. The assembly's concentration was calculated based on the TPPS concentration.

Flow cytometry: Hela cells in fully supplemented DMEM were seeded into 6-well flat-bottom plates 1 × 10^5 cells/well and cultured overnight. Then, cells were treated with PBS, TPPS, TPPS⊂MPCD complex and FACA/TPPS⊂MPCD assembly, respectively, for 24 h ([TPPS] = 10 μM). Then the cells were harvested and washed with PBS for three times and fluorescence data were acquired by flow cytometer (CytoFLEX, Beckman Coulter, USA). The results were analyzed using Flow J software.

Intracellular ROS imaging: The Hela cells were subcultured into a confocal petri dish and incubated for 12 h. Then cells were treated with TPPS, TPPS⊂MPCD complex, FACA/TPPS⊂MPCD assembly, respectively, in the culture medium and cultured for another 24 h ([TPPS] = 10 μM). After this, the culture medium was discarded, and the cells were washed with 0.01 M PBS at least three times. Then the cells were incubated with the commercially available probe 6-carboxy-2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) at 37 °C for 20 min. The cells were repeatedly washed at least three times with PBS. After irradiated at 420 nm for 5 min, the cells were immediately observed by CLSM.

2. Synthesis procedure of target molecules



Scheme S1. Synthesis route of MPCD

4-Propargylmorpholine: 4-Propargylmorpholine was synthesized according to literature[S2] with a little alteration. Morpholine (870.70 mg, 10 mmol) and propargyl bromide (1.30 g, 11 mmol) was dissolved in dry MeCN (80 mL), and K_2CO_3 (2.76 g, 20 mmol) was added in the reaction mixture was stirred at 50 °C under Ar atmosphere for 12 h. Purified by column chromatographic and obtained 840 mg product (yield 67%). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ (ppm): 3.78-3.60 (m, 4H), 3.23 (d, J = 2.5 Hz, 2H), 2.62-2.42 (m, 4H), 2.23 (t, J = 2.4 Hz, 1H).

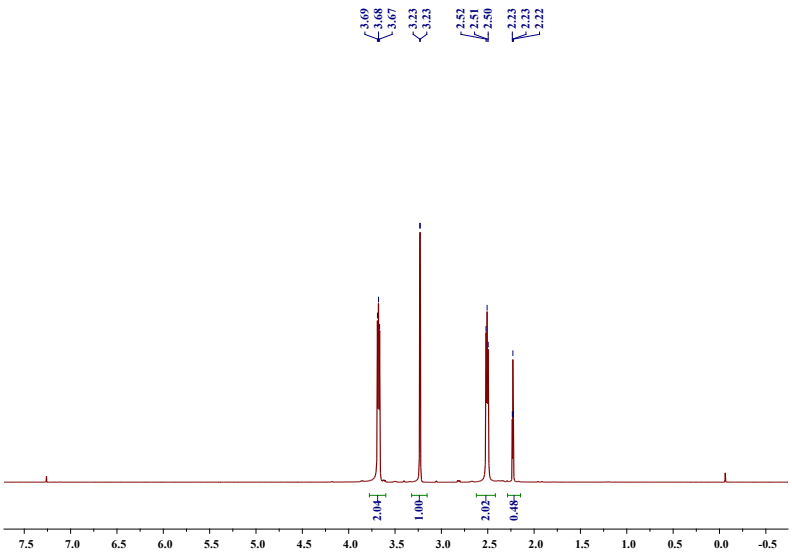


Fig. S1 ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) spectrum of 4-propargylmorpholine.

Synthesis of MPCD: The 4-Propargylmorpholine (75.10 mg, 0.6 mmol) and 6-deoxy-6-azido-permethyl-β-CD (PMCD-N_3) (719.85 mg, 0.5 mmol) were dissolved in anhydrous DMF (10 mL), and iodo(triethyl phosphite)copper(I) (17.83 mg, 0.05mmol) was subsequently added to the solution. The reaction mixture was stirred and reacted at 80 °C under N_2 for 24 h. TLC was employed to monitor the of reaction. After cooled to room temperature, the mixture was filtered to remove any insoluble copper salt. The filtrate was mixed with CH_2Cl_2 (30 mL) and then washed with water (30 mL) to remove DMF and excess unreacted N_3-PMCD. The combined organic phase was dried over anhydrous Na_2SO_4 and evaporated off. The residue was purified by flash chromatography with CH_2Cl_2/MeOH (30:1) as eluent to give MPCD as a white solid (309 mg, yield 39 %). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ (ppm): 7.58 (s, 1H), 5.30 (d, J = 3.1 Hz, 1H), 5.24-5.05 (m, 6H), 4.96-4.69 (m, 2H), 4.11-2.96 (m, 106H), 2.50 (s, 4H). ^{13}C NMR (101 MHz, $CDCl_3$, 25 °C) δ (ppm): 143.5, 125.0, 99.1, 99.0, 98.89, 98.86, 98.7, 98.1, 82.6, 82.2, 82.04, 82.00, 81.95, 81.8, 81.7, 81.5, 81.1, 80.3, 80.0, 79.9, 79.8, 78.7, 77.4, 77.1, 76.7, 71.4, 71.3, 71.1, 70.9, 70.8, 70.6, 70.2, 66.8, 61.7, 61.5, 61.4, 61.3, 59.2, 59.1, 59.0, 58.9, 58.9, 58.6, 58.5, 58.5, 58.4, 58.3, 53.7, 53.4, 51.2. HRMS (ESI): m/z calcd for $C_{69}H_{120}N_4O_{35}$ 1564.7733; $[M+H]^+$ found: 1565.7751.

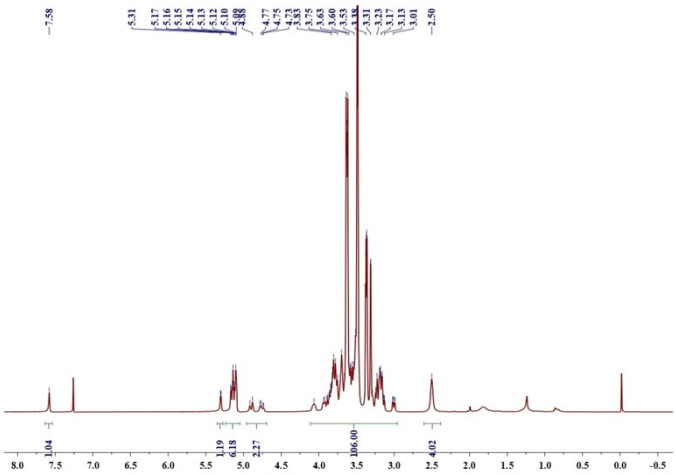


Fig. S2 ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) spectrum of MPCD.

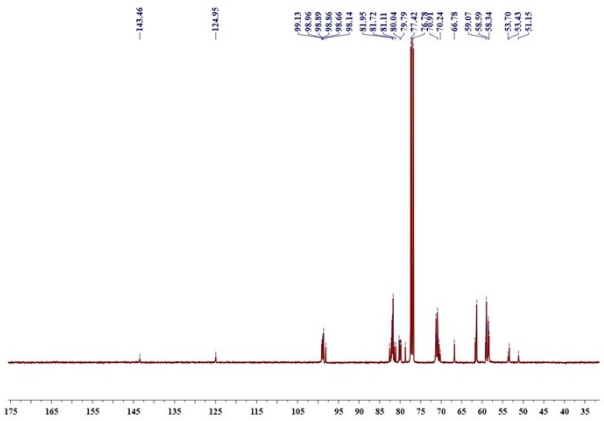


Fig. S3 ^{13}C NMR (101 MHz, $CDCl_3$, 25 °C) spectrum of MPCD.

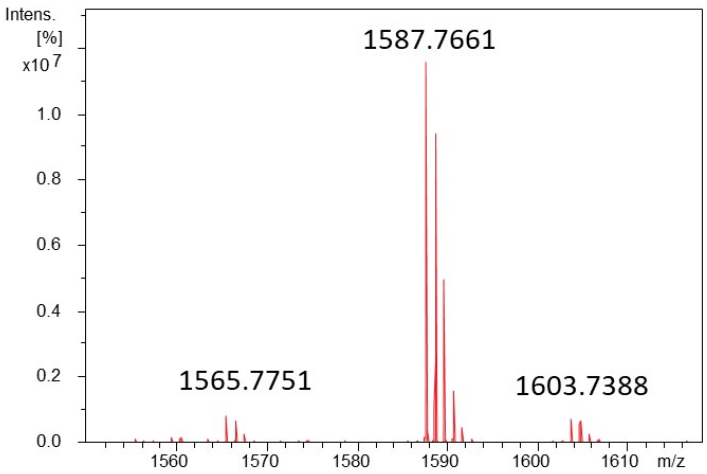
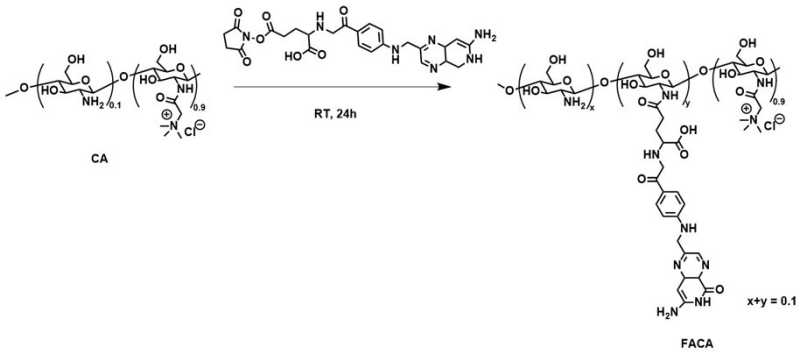


Fig. S4 HRMS of MPCD. The peak at *m*/*z* 1565.7751 corresponds to $[M + H]^+$



Scheme S2. Synthesis route of FACA

Preparation of FACA: FACA was prepared according to the reported literature[S3] with a little modification. Folic acid (441.40 mg, 1 mmol) was activated with *N*-hydroxysuccimide (115.09 mg, 1 mmol) and dicyclohexylcarbodiimide (206.33 mg, 1.5mmol) at 50 °C for 6 h. The precipitate was removed by filtration (0.22 μm) and the NHS-FA was precipitated by acetone and ether (V/V = 3:7). The activated FA (300 mg) was reacted with aqueous solution of CA (4 mg/mL, 50 mL) at room temperature for 24 h. The mixture was dialyzed (MWCO 8000) against deionized water and was centrifuged (8000 rpm, 10 min) to remove excess NHS-FA. The appearance of the proton signals in the downfield of the ^{1}H NMR spectrum and the molecular vibration peaks in the FT-IR spectrum jointly indicate the successful modification of folic acid groups on the backbone of chitosan.

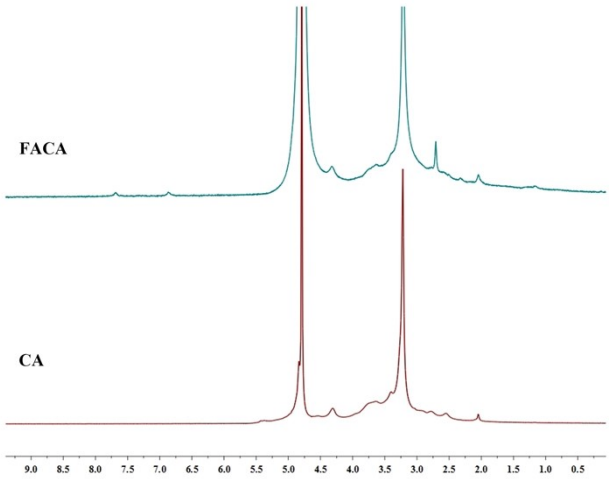


Fig. S5 ^{1}H NMR (400 MHz, D_2O, 25 °C) spectra of CA and FACA.

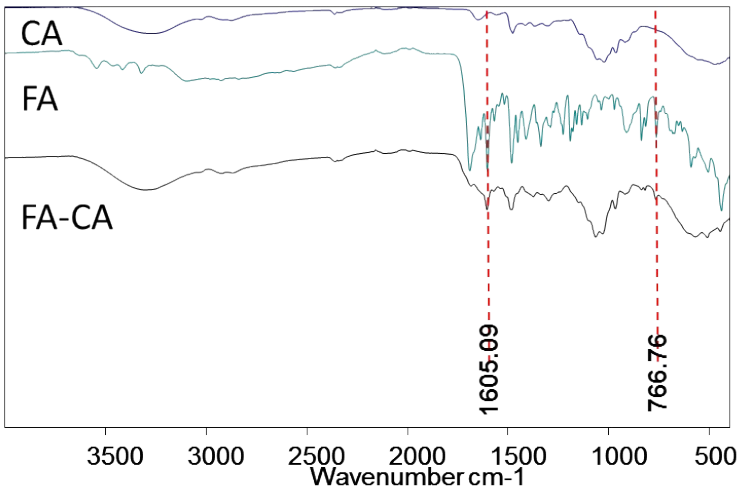


Fig. S6 FT-IR spectra of CA, FA and FACA.

3. ^{1}H NMR spectra of MPCD and TPPS

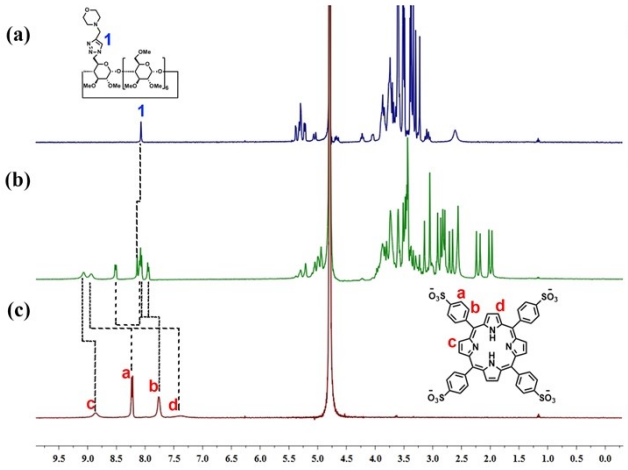


Fig. S7 ^{1}H NMR spectra (400 MHz, D_2O, 25 °C) of (a) MPCD, (b) TPPS⊂MPCD complex, and (c) TPPS ([MPCD] = 10 mM, [TPPS] = 5 mM).

4. 2D NOESY spectrum of MPCD and TPPS

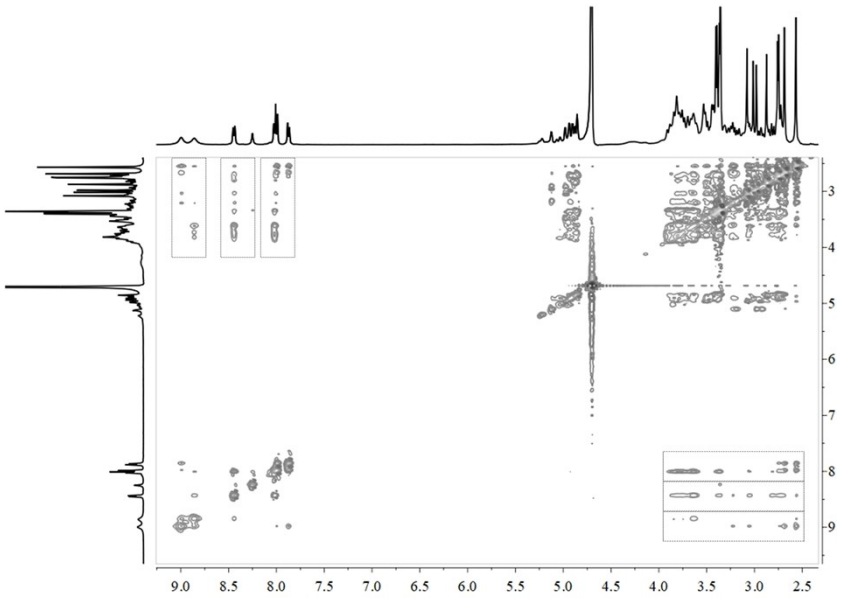


Fig. S8 2D NOESY spectrum (400 MHz, D_2O, 25 °C) of MPCD and TPPS. ([MPCD] = 10 mM, [TPPS] = 5 mM)

5. Job plot and binding constant for MPCD and TPPS

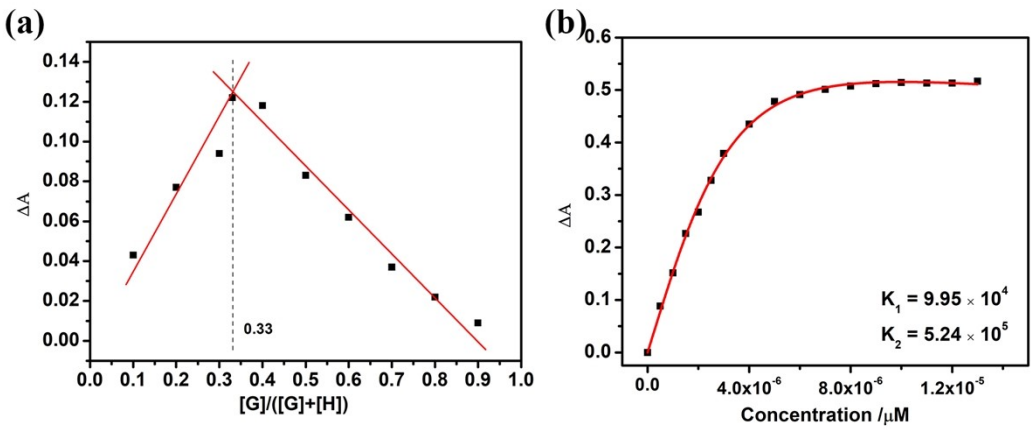


Fig. S9 (a) Job plot showing 2:1 stoichiometry of the complexation between MPCD and TPPS at 414 nm in PBS (7.2, 0.01 M). ([MPCD] + [TPPS] = 6 μM) and (b) The nonlinear curve fitting of the variation of UV-Vis absorbance intensity with the concentration of MPCD to calculate the association constant at 414 nm in PBS (pH 7.2, 0.01 M, [TPPS] = 5 μM).

6. UV-Vis absorbance spectrum of TPPS with MPCD

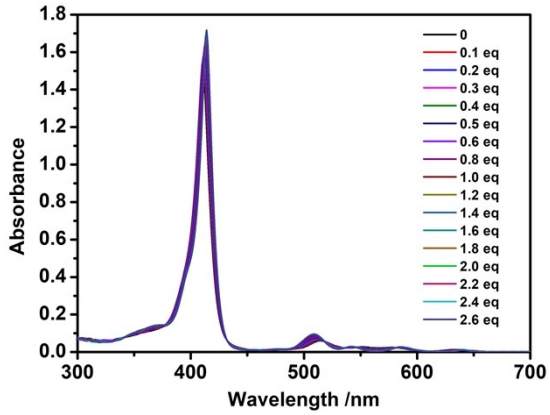


Fig. S10 UV-Vis absorbance spectra of TPPS with MPCD ([TPPS] = 5 μM). The concentrations of MPCD were 0, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 μM, respectively, at pH = 7.2 (0.01 M PBS)

7. Fluorescence emission spectra of adding MPCD to TPPS

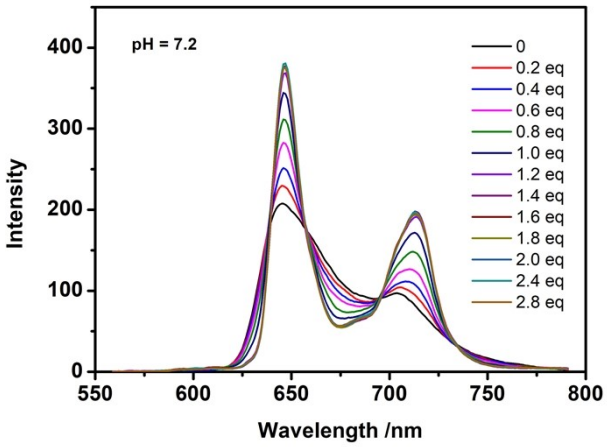


Fig. S11 Fluorescence emission spectra of TPPS with MPCD ([TPPS] = 10 μM). The concentrations of MPCD were 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 24, 28 μM, respectively, at pH = 7.2 (0.01 M PBS).

8. UV-Vis absorbance spectra for adding MPCD to TPPS at different pH values

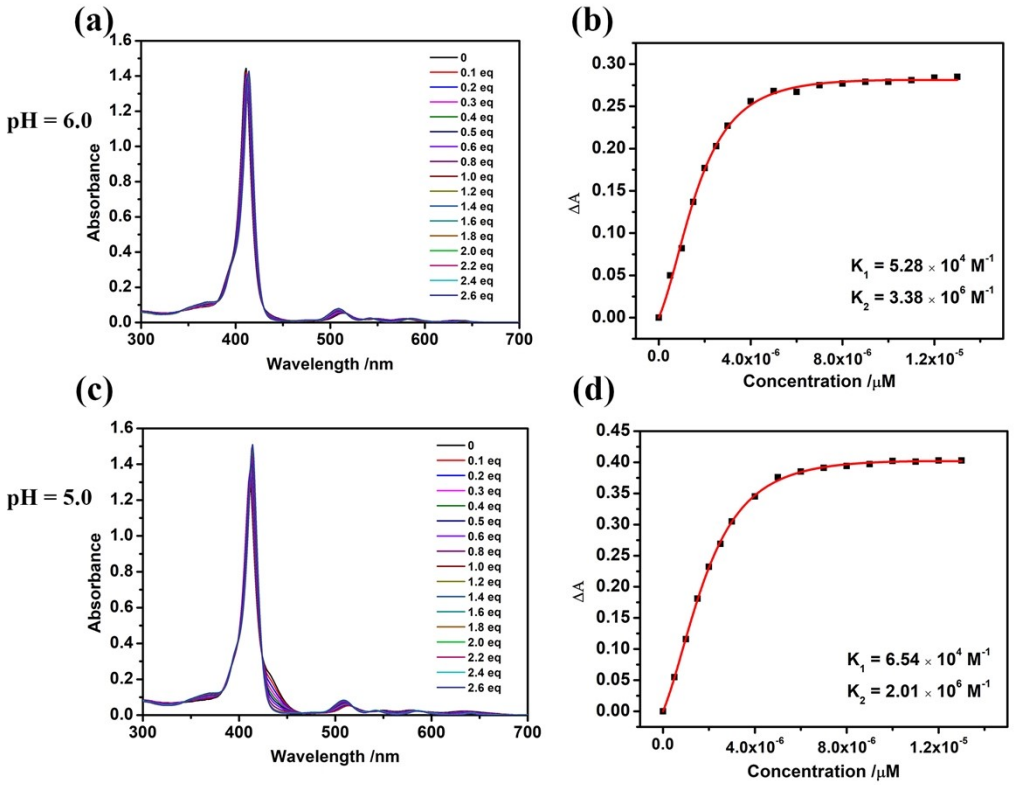


Fig. S12 UV-Vis absorbance spectra of TPPS with MPCD ([TPPS] = 5 μM). The concentrations of MPCD were 0, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 μM, respectively, at (a) pH = 6.0 (0.01 M PBS) and (c) pH = 5.0 (0.01 M PBS). The nonlinear curve fitting of the variation of UV-Vis absorbance at 414 nm with the concentrations of MPCD to calculate the binding constants at (b) pH 6.0 and (d) pH 5.0.

9. Fluorescence spectra of TPPS with MPCD in acidic environment

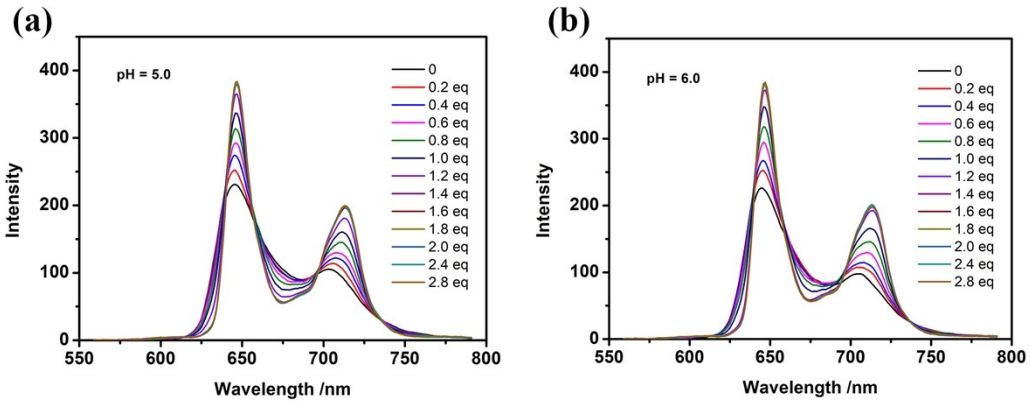
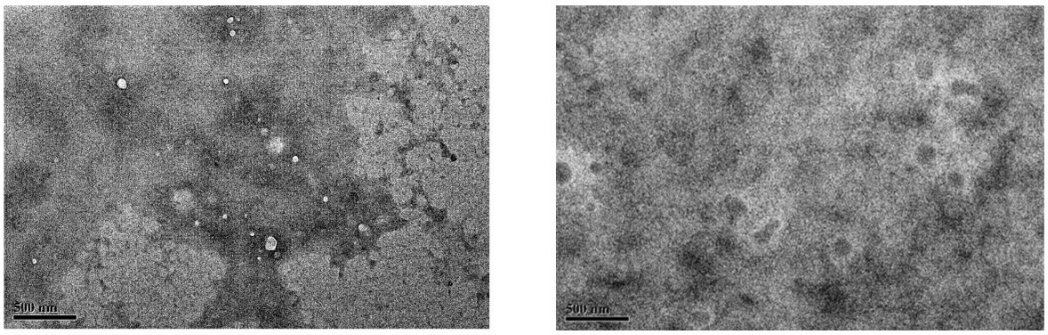


Fig. S13 Fluorescence emission spectra of TPPS with MPCD ([TPPS] = 10 μM). The concentrations of MPCD were 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 24, 28 μM, respectively, at (a) pH = 6.0 (0.01 M PBS) and (b) pH = 5.0 (0.01 M PBS).

10. TEM images of TPPS⊂MPCD complex and FACA



TPPS⊂MPCD **FACA**

Fig. S14 TEM images of TPPS⊂MPCD complex and FACA.

11. TEM images FACA and TPPS⊂MPCD complex at different concentrations

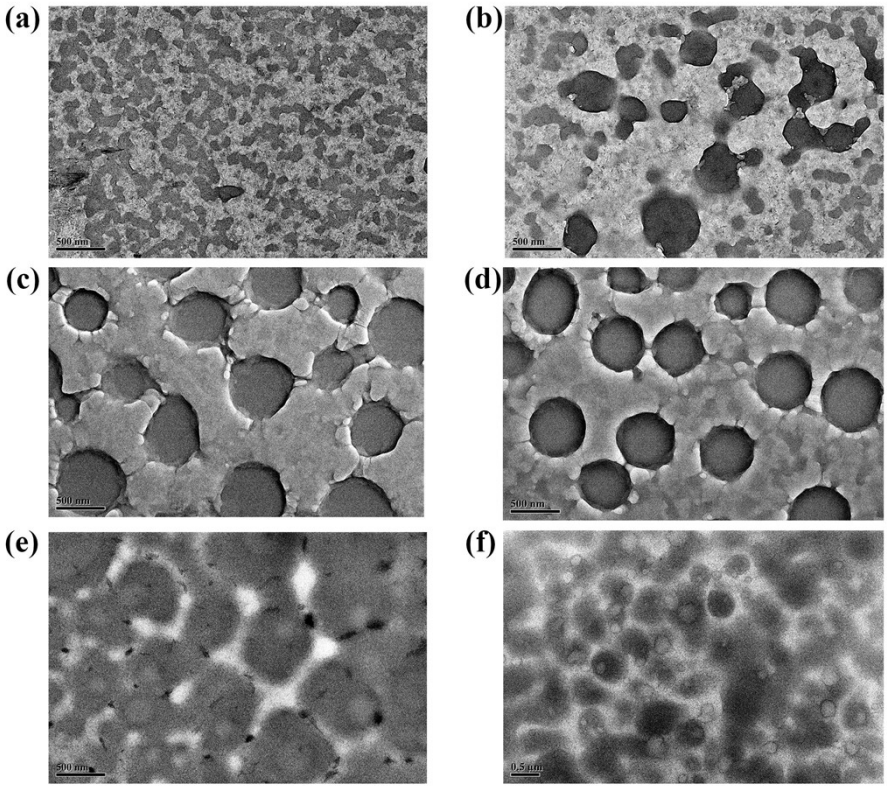


Fig. S15 TEM images of TPPS⊂MPCD complex ([TPPS] = 4×10^{-5} M and [MPCD] = 8×10^{-5} M) with different concentrations of FACA (a) 1×10^{-5} mg/mL, (b) 2×10^{-5} mg/mL, (c) 4×10^{-5} mg/mL, (d) 6×10^{-5} mg/mL, (e) 1×10^{-4} mg/mL, and (f) 1.5×10^{-4} mg/mL.

12. Tyndall effect images of FACA, TPPS⊂MPCD and FACA/TPPS⊂MPCD assembly

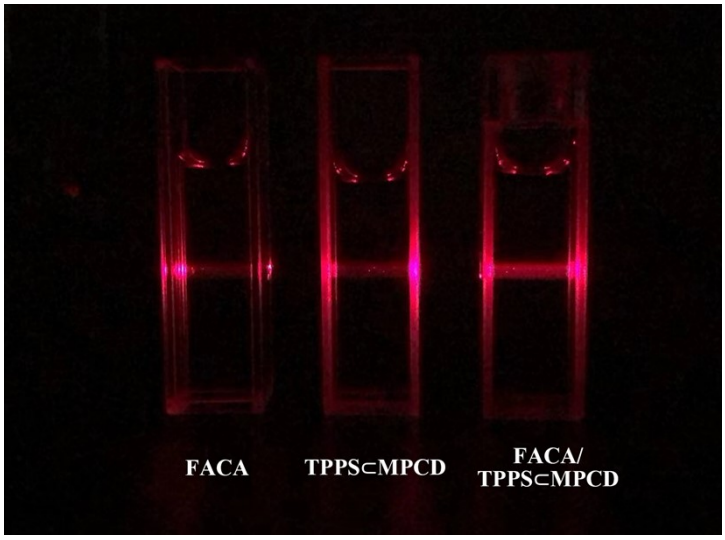


Fig. S16 Images of Tyndall effect of FACA, TPPS⊂MPCD complex and FACA/TPPS⊂MPCD assembly.

13. UV-Vis absorbance spectra for adding FACA to TPPS and TPPS⊂MPCD complex

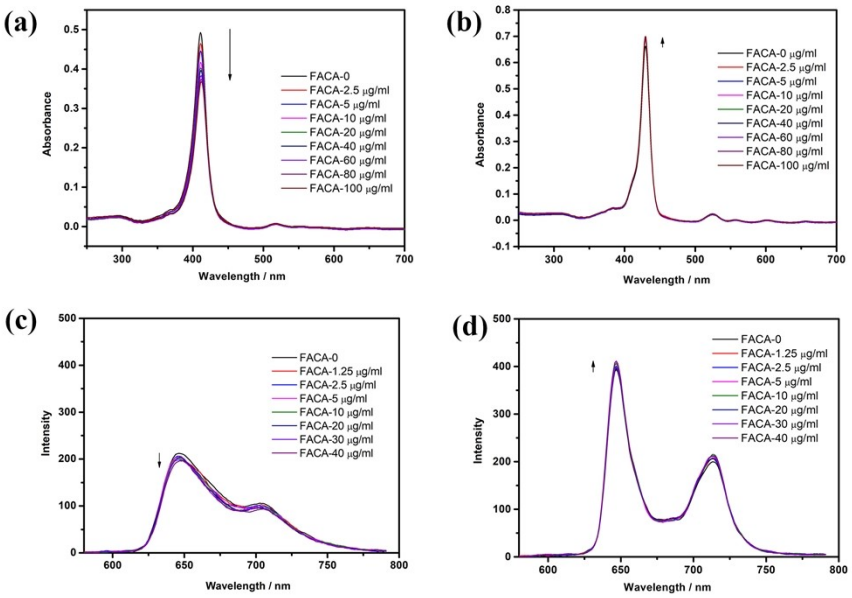


Fig. S17 (a) UV-Vis absorbance spectra of (a) TPPS and (b) TPPS⊂MPCD complex upon addition of FACA (0-100 μg/mL) in PBS (0.01 M) at 25 °C ([TPPS] = 3 μM); fluorescence emission spectra of (c) TPPS and (d) TPPS⊂MPCD complex upon addition of FACA (0-40 μg/mL) in PBS (pH 7.2, 0.01 M) at 25 °C. ([TPPS] = 10 μM).

14. ^{1}H NMR spectra of FACA and TPPS⊂MPCD complex

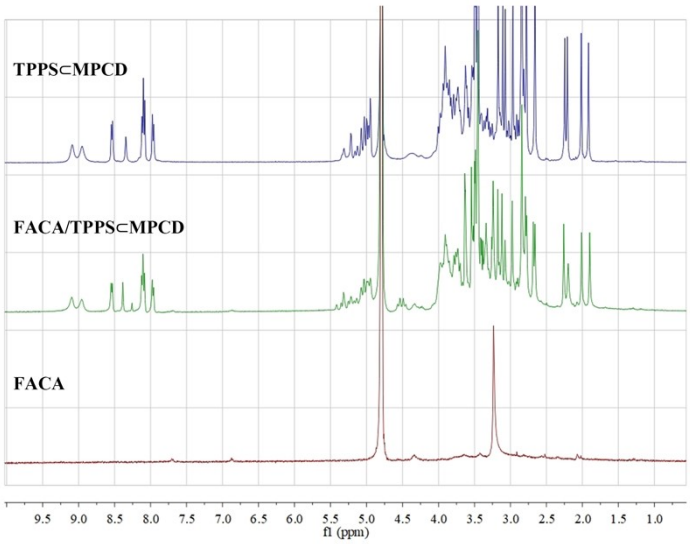


Fig. S18 ^{1}H NMR spectra (400 MHz, D_2O, 25 °C) of (top) TPPS⊂MPCD complex, (middle) FACA/TPPS⊂MPCD assembly and (bottom) FACA ([MPCD] = 10 mM, [TPPS] = 5 mM, and [FACA] = 5 mg/mL).

15. 2D NOESY spectrum of FACA/TPPS⊂MPCD assembly

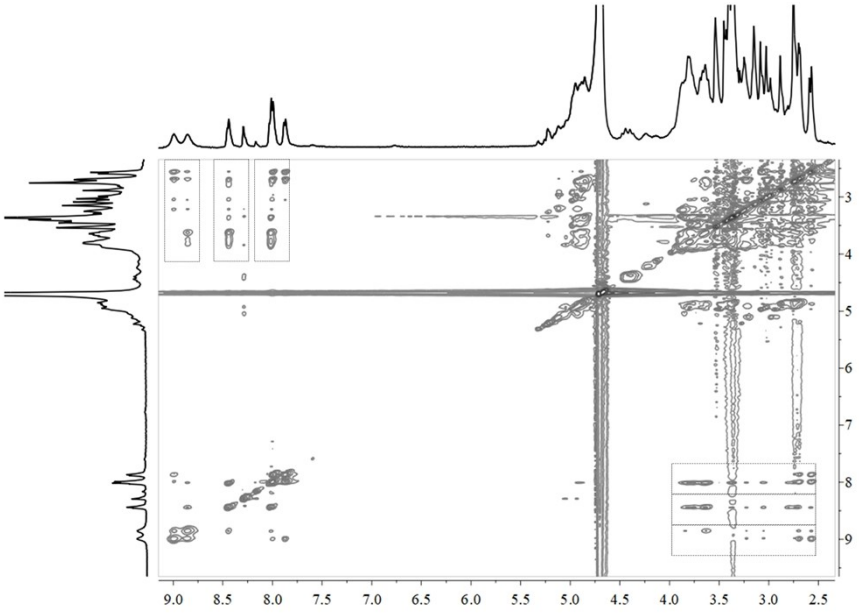


Fig. S19 2D NOESY spectrum (400 MHz, D_2O, 25 °C) of FACA/TPPS⊂MPCD assembly ([MPCD] = 10 mM, [TPPS] = 5 mM, and [FACA] = 5 mg/mL).

16. ROS generation at different pH values

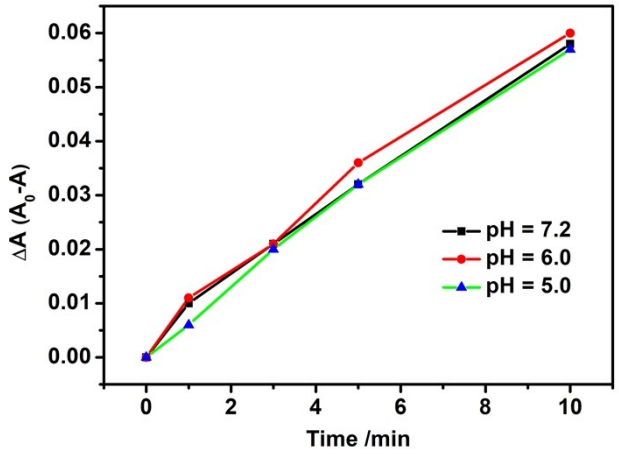


Fig. S20 Decomposition rate of ABDA at 378 nm versus different irradiation time in different pH. A_0 is the original absorption of ABDA, and A is the absorption with different irradiation time. [TPPS] = 5 μM.

17. Pearson correlation coefficient of FACA/TPPS⊂MPCD assembly

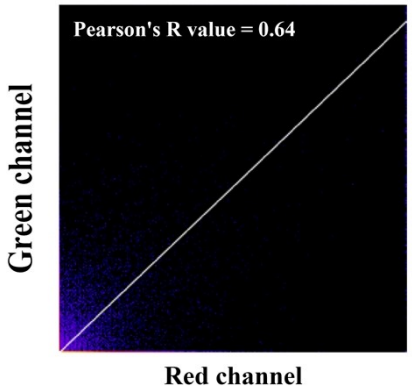


Fig. S21 Pearson correlation coefficient of FACA/TPPS⊂MPCD assembly.

18. CLSM images of FACA/TPPS⊂PCD assembly

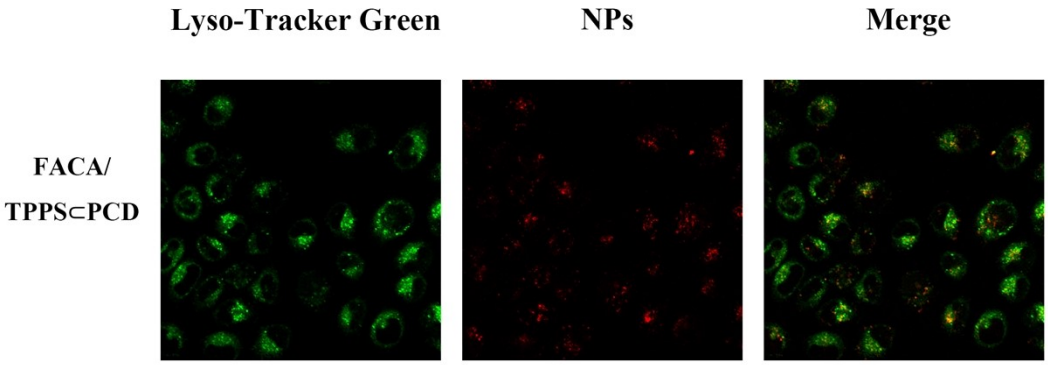


Fig. S22 Lysosome colocalization CLSM images in living Hela cancer cells treated with FACA/TPPS⊂PCD assembly

19. Pearson correlation coefficient of FACA/TPPS⊂PCD assembly

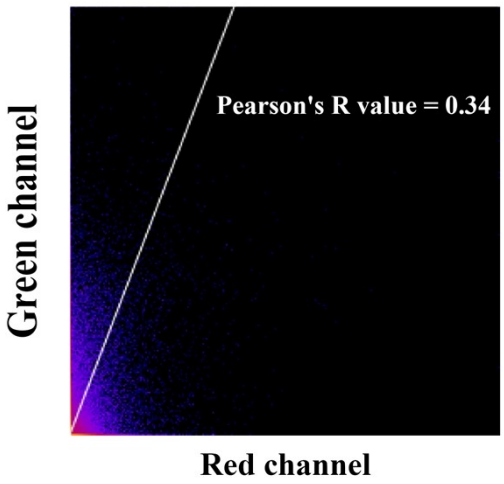


Fig. S23 Pearson correlation coefficient of FACA/TPPS⊂PCD assembly.

20. Side effect to normal cells

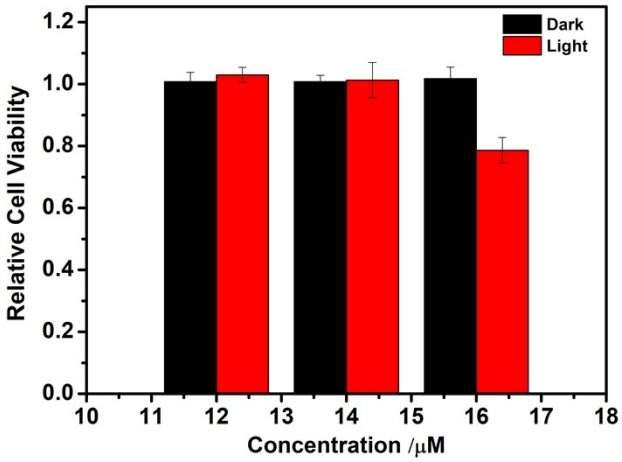


Fig. S24 In vitro cell viability of MRC-5 cells after being treated with FACA/TPPS⊂MPCD assembly at different concentrations for 24 h. The concentrations were calculated based on concentration of TPPS.

References

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