Electronic Supplementary Information

Water-soluble Fluorescent Unimolecular Micelles: Ultra-small Size, Tunable Fluorescence Emission from Visible to NIR Region and Enhanced Biocompatibility for In Vitro and In Vivo Bioimaging

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1. General Methods

1.1 Materials

All chemicals including methacryloyl chloride (MA), 1, 6-hexanediol, 5(6)-Carboxyfluorescein diacetate (CFD), 5(6)-Carboxytetramethylrhodamine (CTR) dicyclohexylcarbodiimide (DCC), 4-dimethylaminopyridine (DMAP), poly (ethylene glycol) methyl ether methacrylate (OEGMA), tris[2-(dimethylamino)ethyl]amine (Me_6TREN), copper(I) bromide (CuBr) and anhydrous solvent including tetrahydrofuran (THF), dimethyl sulfoxide(DMSO) and *N, N*-Dimethylformamide (DMF) were purchased from Sigma-Aldrich (USA). Cy5 NHS ester (NHS-Cy5) was purchased from RuiXi Biological Technology Co., Ltd. The other solvents were analytical grade and were purchased from Adamas-beta® (China). All biological reagents including Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), Alexa Fluor®633 phalloidin (AF-633), 4'6-diamidino-2-phenylindole (DAPI), TrypLE™ Express Enzyme, PrestoBlue, LysoTracker@Red, LysoTracker@Green and Mito-Tracker@Green were purchased from Life Technologies (China).

1.2 Instruments

All of the nuclear magnetic resonance (NMR) spectra were obtained on a BRUKER Avance 600 NMR spectrometer using tetramethylsilane ($\delta = 0$) as internal reference, the solvent was deuterated methanol (CD_4O) or $CDCl_3$. Ultraviolet (UV)−vis spectra were acquired on a Shimadzu UV-1800 spectrophotometer. Fluorescence spectra were detected on a Shimadzu RF-5301PC fluorescence spectrometer. The morphology based on TEM images were acquired by a JEM-1230EX transmission electron microscopy (TEM). The DLS results were performed on a Malvern Nano ZS90. Fourier transform infrared (FT-IR) spectra were detected using a Thermo Nicolet 6700 FT-IR spectrophotometer. The molecular weight (Mw) and molecular weight

distribution (Mw/Mn) were tested by gel permeation chromatography (GPC) of Agilent 1260， where containted a 1260 pump, a refractive index detector and a styragel®HT column. Tetrahydrofuran and polystyrene were selected as eluent solvent and standard samples, respectively. Fluorescence images of cells were carried out on a confocal laser scanning microscopy of Zeiss 800. Flow cytometry was obtained on account of a NovoCyte 2060R. The complete blood test was tested based on a Mindray BC-2600Vet haematology analyser. The quantum yield of PCFD, PCTR and PCy5 UMs in water were measured by employing Rhodamine B in ethanol (Φ_F = 0.95) as reference substance.[1]

1.3 Synthesis of PCFD copolymer

Synthesis of MAHDO. Typically, a mixture of 1, 6-hexanediol (6.25g, 0.05mol), trimethylamine (3.3mL, TEA) and tetrahydrofuran (THF, 50 mL) were added into a Schlenk tube (100 mL) under argon atmosphere. Then 15mL THF containing 2.2mL methacryloyl chloride was dropwise added into above solution under ice bath. After being stirred at room temperature for 12 h, the resulted solutions were collected and extracted by HCl (0.3M), NaOH (0.3M) and saturation salt water orderly. Finally, the product was purified by passing through silica column chromatography (Eluent: hexane and ethyl acetate (EA), volume/volume=4:1), yielding a yellow product (denoted as MAHDO). (1.72g, 42.5 % yield). ^{1}HNMR (600MHz, $CDCl_3$, δ, ppm, TMS, Figure S4):6.05 (s, 2H), 5.50 (s, 2H), 4.18 (t, 2H), 4.18-4.11(t, 2H), 1.88 (s, 3H), 1.70-1.68 (q, 2H), 1.59-1.57 (t, 2H), 1.44-1.39 (m, 4H).

Synthesis of MCFD monomer. In briefly, a mixture of 5(6)-Carboxyfluorescein diacetate (CFD) (95.2mg, 0.2mmol), DCC (51mg, 025mmol) and chloroform (3 mL) were added into a Schlenk tube (10 mL) under argon atmosphere. After being stirred at ice bath for 0.5 h, the mixture of MAHDO (57mg, 0.3mmol), 4-dimethylaminopyridine (DMAP) (30mg, 0.25mmol) and chloroform (0.5 mL) was added and stirred for another 0.5h under 0 ^{o}C, and then stirred for 48h at 25 ^{o}C. Then the collected solution was

diluted by dichloromethane (DCM) and purified by extraction, including deionized water, 0.1 M HCl and saturation salt water, orderly. Finally, the product was purified by passing through a silica column chromatography (Eluent: methanol and DCM, volume/volume=1:20), resulting a yellow product of MCFD (83.2mg, 64.3 % yield). ^{1}HNMR (600MHz, $CDCl_3$, δ, ppm, TMS, Figure S5A): 8.32-8.26 (m, 1H), 8.12-8.03 (m, 1H), 7.83-7.72 (m, 1H), 7.14-7.06 (m, 1H), 6.88-6.78 (m, 1H), 6.69-6.52 (m, 1H), 6.12-6.04 (t, 1H), 5.57-5.51(d, 1H), 4.18-4.12 (m, 2H), 4.09-4.02 (t, 2H), 2.33 (s, 2H), 1.98-1.89 (d, 3H), 1.66-1.53 (m, 2H), 1.46-1.38 (m, 2H), 1.26 (s, 4H). MS (ESI): calcd. for $C_{36}H_{36}O_{10}$, $[M+H]^+$ m/z 629.23, found 629.45. FTIR (KBr cm^{-1}): ν = 3327.28, 2928.28, 2850.92, 1720.66, 1627.11, 1575.48, 1450.77, 1311.32, 1273.06, 1243.06, 1167.90, 1111.59, 435.68.

Synthesis of β-CD-P(MCFD-co-OEGMA) (PCFD) copolymer. The amphiphilic copolymer of PCFD was synthesized by atom transfer radical polymerization (ATRP). Firstly, β-CD-Br was prepared with the reported method as the macroinitiator.[2] Next, a mixture β-CD-Br (12mg, 0.0025mmol), MCFD (123mg, 0.20mmol), Poly (ethylene glycol) methyl ether methacrylate (OEGMA, 100mg, 0.2mmol), CuBr (8 mg, 0. 05mmol) and 3 mL DMSO were dispersed into a Schlenk tube (25 mL) under argon atmosphere. After three freeze−pump−thaw cycles by liquid nitrogen being proceed, Me_6TREN (15.0 uL, 0.05 mmol) was injected into the above solution using the micro-syringe. The ATRP reaction was conducted at 25 °C by stirring for 48 h and then the resulted solution was dialyzed using methanol to remove unreacted monomer and solvent. After the solvent being concentrated, a viscous solid denoted as β-CD-P(MCFD-*co*-OEGMA) (PCFD) was obtained (106.6mg, 45.3 % yields). ^{1}HNMR (600MHz, $CDCl_3$, δ, ppm, TMS, Figure S5B): 8.36-8.20 (br, 1H), 8.13-7.89 (t, 1H), 7.85-7.69 (d, 1H), 7.15-7.01 (d, 1H), 6.88-6.69 (t, 1H), 6.64-6.45 (d, 1H), 4.2-3.95 (s, 4H), 3.66 (s, 2H), 3.34 (s, 3H), 2.29 (s,3H), 2.09-1.95 (d, 3H), 1.69-1.47 (s, 2H), 1.44 (s, 2H), 1.24(s, 4H). FTIR (KBr cm^{-1}): ν =2918.99, 1729.35, 1249.41, 1109.82.

1.4 Synthesis of PCTR copolymer

Synthesis of MCTR monomer. Typically, a mixture of DCC (51mg, 025mmol), 5(6)-carboxytetramethylrhodamine (CTR) (86.09mg, 0.2mmol) and anhydrous DMF (3 mL) were added into a Schlenk tube (10 mL) under argon atmosphere. After being stirred at ice bath for 0.5 h, the mixture of MAHDO (57mg, 0.3mmol) and DMAP (30mg, 0.25mmol) was added and stirred for another 0.5h under 0 ^{o}C, and then stirred for 48h at 25 ^{o}C. Then the collected solution was diluted by DCM and purified by extraction, including deionized water, 0.1 M HCl and brine, respectively. Finally, the product was purified by passing through silica column chromatography (Eluent: methanol and DCM, volume/volume=1:20), yielding a solid product of MCTR (73.8mg, 60.1 % yields). ^{1}HNMR (600MHz, CD_4O, δ, ppm, TMS, Figure S6A): 8.31-8.23 (d, 1H), 8.18-8.08 (d, 1H), 7.95-7.83 (t, 1H), 7.52-7.42 t, 1H), 7.32-7.20 (d, 1H), 7.16-6.97 (m, 2H), 6.07 (s, 1H), 5.60 (s, 1H), 4.45-4.24 (m, 2H), 4.16-4.07 (t, 2H), 3.56-3.44 (m, 12H), 1.98-1.79 (m, 3H), 1.79-1.58 (br, 2H), 1.56-1.30 (br, 2H), 1.28-1.09 (m, 4H). MS (ESI): calcd. for $C_{35}H_{39}N_2O_7$, $[M]^+$ m/z 599.28, found 599.55. FTIR (KBr cm^{-1}): ν =2933.71, 1717.62, 1321.55, 1297.80, 1166.64.

Synthesis of β-CD-P(MCTR-co-OEGMA) (PCTR) copolymer. Specifically, β-CD-Br (12mg, 0.0025mmol), MCTR (123mg, 0.20mmol), OEGMA (100mg, 0.2mmol), CuBr (8 mg, 0. 05mmol) and 3 mL DMSO were placed in a Schlenk tube (10mL) under argon atmosphere. The Schlenk tube was then sealed, degassed, and refilled with argon gas through three freeze−pump−thaw cycles. Me_6TREN (15.0 uL, 0.05 mmol) was then injected into the above solution using the micro-syringe. The ATRP reaction was conducted at 25 °C by stirring for 48 h. The resulted solution was dialyzed using methanol to remove unreacted monomer and solvent. After being concentrated, a final product denoted as β-CD-P(MCTR-*co*-OEGMA) (PCTR) was obtained (97.3mg, 41.4 % yields). ^{1}HNMR (600MHz, CD_4O, δ, ppm, TMS, Figure S6B): 8.01-6.97 (d, 9H), 3.65 (s, 2H), 3.36 (s, 3H), 3.29 (s, 12H), 1.14-1.77 (t, 3H), 1.76-1.52 (d, 2H), 1.43 (s, 2H), 1.37-1.01 (br, 4H). FTIR (KBr cm^{-1}): ν =3440.97, 2921.32, 1728.69, 1105.84.

1.5 Synthesis of PCy5 copolymer

Synthesis of 2-((tert-butoxycarbonyl) amino) ethyl methacrylate (MMEA). Specifically, ethanol amine (611mg, 10 mmol) and di-tert-butyl dicarbonate (2.18 g) were dissolved in 30 mL of acetonitrile and the solution was stirred for 15 min. The solvent was then removed by evaporation, produced a product of tert-butyl-N-(2-hydroxyethyl) carbamate for immediate reaction. Next, tert-butyl-N-(2-hydroxyethyl) carbamate (10 g, 62 mmol) and *N, N*-diisopropylethylamine(16.2 mL, 93 mmol) were dissolved in 100 mL of DCM, the mixture was stirred for 24 h at 25 ^{o}C. The final product was purified by passing through silica column chromatography (Eluent: DCM and hexane, volume/volume=1:10). The residue was collected by evaporation and then dried under vacuum to obtain the product of MMEA. ^{1}HNMR (600MHz, $CDCl_3$, δ, ppm, TMS, Figure S7): 6.12 (s, 1H), 5.58 (s, 1H), 4.18 (s, 2H), 3.44 (s, 2H), 1.95 (s, 3H), 1.43 (s, 9H).

Synthesis of β-CD-P(MMEA-co-OEGMA) copolymer. A mixture of β-CD-Br (48mg, 0.01mmol), MMEA (96mg, 0.40mmol), OEGMA (400mg, 0.8mmol) , CuBr (32 mg, 0. 2mmol) and 3 mL DMSO were placed in a Schlenk tube (10mL) under argon atmosphere. The Schlenk tube was then sealed, degassed, and refilled with argon gas through three freeze−pump−thaw cycles. Me_6TREN (15.0 uL, 0.05 mmol) was then injected into the above solution using the micro-syringe. The ATRP reaction was conducted at 25 °C by stirring for 48 h. Then Al_2O_3 column was used to remove the copper catalyst. The resulted solution was precipitated using diethyl ether and dried under vacuum to obtain the final product (223.1mg, 40.3 % yields). ^{1}HNMR (600MHz, $CDCl_3$, δ, ppm, TMS, Figure S8A):4.43-8.38 (br, 2H), 3.60 (s, 2H), 3.50 (s, 2H), 3.34 (s, 3H), 2.08-1.60 (br, 3H), 1.42 (s, 9H).

Synthesis of β-CD-P(MCy5-co-OEGMA) (PCy5) copolymer. Firstly, β-CD-P(MMEA-*co*-OEGMA) (60 mg) was dissolved in the mixture solvent of 5mL DCM and 5mL trifluoroacetic acid (TFA), and the resulted solution was stirred for 0.5 h. After the solvent being evaporated, the obtained product was further dissolved in 2 mL of

DMF containing 20uL trimethylamine (TEA) and then NHS-Cy5 (5.0 mg) was added. The mixture was stirred for 72 h under dark and then dialyzed using methanol for 48 h. After removing the methanol by evaporation, a viscous blue solid (denoted as PCy5) was obtained (17 mg, 32.1% yields). ^{1}HNMR (600MHz, $CDCl_3$, δ, ppm, TMS, Figure S8B): 8.64-7.65 (m, 8H), 4.08 (s, 2H), 3.68 (s, 2H), 3.57 (s, 2H), 3.39 s, 3H), 2.1-1.67 (s, 9H), 1.48-0.96 (m, 6H). FTIR (KBr cm^{-1}): ν =3591.82, 3562.52, 3466.17, 2919.05, 1728.96, 1457.77, 1105.65.

1.6 Preparation of unimolecular micelles (UMs)

Firstly, 5.0 mg of PCFD, PCTR or PCy5 copolymer were dissolved in 0.5 mL of DMF and were dispersed in 8 mL of DI water. The solution was then dialyzed by DI water (2 L×3) for 24 h using a dialysis bag (MWCO=3500) to remove DMF, the resulted UMs were obtained and were diluted to 200 μg/mL for following experiments.

1.7 Photobleaching study

To investigate the photobleaching of PCFD, PCTR and PCy5 UMs in water medium, 2 mL of UMs were added in cuvette and then placed in a black box with irradiation of power-adjustable LED (wavelength was 470 nm, 12 W). The distance from LED source to cuvette was adjusted to 10 cm, and then the fluorescence intensity was recorded at different time points (0, 1, 2, 4, 6 and 12 h) by fluorescence spectrophotometer (Shimadzu RF-5301PC).

1.8 Cell culture and animal study

HeLa immortal cancer cells and L929 mouse fibroblast cells were provided from the Shanghai Cell Bank of the Chinese Academy of Sciences, China. These cells were incubated in Dulbecco's modified Eagle's medium (DMEM) containing 1% penicillin and 10% fetal bovine serum (FBS) at 37 °C under 5% CO_2. The animal study was in

compliance with the National Guide for Care and Use of Laboratory Animals, and the experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of Southwest University. Sprague Dawley (SD) mice (4 weeks, 200-220 g) and BALB/c nude mice (5-6 weeks, female, and 18-20 g) were brought from Tengxin Biology Company (Chongqing, China).

1.9 In vitro cytotoxicity

The cytotoxicity of PCFD, PCTR or PCy5 UMs against HeLa and L929 cells were evaluated using Prestoblue assay. Typically, HeLa and L929 cells were incubated with DMEM containing 10 % FBS and 1 % penicillin/streptomycin (P/S) with 5 % CO_2 at 37 °C. Next, 1×10^4 cells and 200 μL DMEM were put in each well of 96-well plates, respectively. After being incubated for 12 h, the DMEM was poured out. The cells were washed with fresh PBS and refilled with fresh DMEM containing different concentrations of PCFD, PCTR or PCy5 micelles ranging from 0 to 50 ug/mL. After being incubated for 72 h, the dye solution was discarded and the DMEM solution of PrestoBlue reagent was added into each well. After being incubated for 1h, the absorbance at 570 nm and 600 nm were detected by a Plate Reader of Tecan Spark-10. The cytoactive of cells without treatment were acted as 100 % and served as control.

1.10 *In vitro* fluorescent imaging

HeLa cells were seeded in 8-well plates with a density of 5×10^4 per well at 37 °C, After 80 % confluence, the medium was replaced with fresh one and incubated with PCFD (20 μg/mL), PCTR (20 μg/mL) or PCy5 (20 μg/mL) UMs. The fluorescence images of cells were recorded by confocal laser scanning microscope (CLSM, Zeiss 800, Germany). Alexa Fluor® 633 phalloidin (AF-633), Mito-Tracker@Green, Lyso Tracker@Red or LysoTracker@Green, DAPI were used to stain the cell membrane, mitochondria, lysosome, nucleus, respectively. The lasers of 495nm, 558nm, 650 nm, 631 nm, 490nm, 561 nm, 503nm and 405 nm were used to excite PCFD, PCTR,

PCy5, Alexa Fluor® 633 phalloidin, Mito-Tracker@Green, Lyso Tracker@Red, Lyso Tracker@Green and DAPI, respectively. The corresponding fluorescence emissions were recorded by CLSM in the range of 455-550nm, 570-614nm, 650-700 nm, 645-700 nm, 450-525nm, 566-670 nm, 490-565nm and 410-480 nm for imaging in the six channels (Objective: EC Plan-Neofluar 20x/0.30 M27; dimension is 4096 ×4096).

1.11 *In vitro* cellular uptake

HeLa cells were preincubated in 6-well plates at 37 °C with 5 % CO_2 for 12h, PCFD UMs and PCy5 UMs (Final concentration: 20ug/mL) were introduced into the medium and were incubated with HeLa cells for 2h and 6h. The medium was then discarded and rinsed with PBS for three times. 1.0 mL TrypLE™ Express Enzyme was added to detach the cells and the culture medium was used to stop trypsinization. Upon a centrifugation, the suspension liquid was diluted with 0.5 mL PBS for flow cytometry (NovoCyte 2060R). The fluorescence of PCFD and PCy5 were detected using PE (excitation wavelength: 488 nm, emission wavelength: 575 nm) and APC-Cy7 (excitation wavelength: 620-650nm, emission wavelength: 750 nm) channels, with ~1×10^5 gated cells. The cells without any fluorescence staining were used as the control. The data were analyzed by FlowJo software.

1.12 *In vivo* complete blood count

The blood compatibility of PCFD, PCTR and PCy5 UMs were tested by the tail vein injection on the mouse model. The dosage of dye injected was 20 mg/kg. The selected SD mice had average weight of approximately 200 g and were randomly divided into four groups (n=3 per group). 2 mL of dye UMs (Final concentration: 2 mg/mL) were injected through tail vein. A group of mice were injected with 2 mL PBS without any dye UMs as the control. After 7 days, 500 μL of blood was collected in a sample tube with EDTA from each mouse for hematology analysis. The data embracing white blood

cell count (WBC), lymphocyte ratio (Lymph%), red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular hemoglobin concentration (MCHC),red cell distribution width (RDW), platelet (PLT) and mean platelet volume (MPV) were mainly analyzed.

1.13 *In vivo* imaging

After nude mice bearing MCF-7 tumors were anesthetized, free Cy5 and PCy5 UMs with equivalent concentration of Cy5 (1.0 mg/kg) were systematically injected through the tail vein using a microsyringe. Fluorescence whole animal imaging was performed using a NIR imaging system (PerkinElmer IVIS Lumina Kinetic Series III) at 650 nm. The fluorescence distribution was monitored at 24 h after Cy5 administration. The *in vivo* fluorescence with excited light from 610 to 800 nm were recorded from the tumor regions.

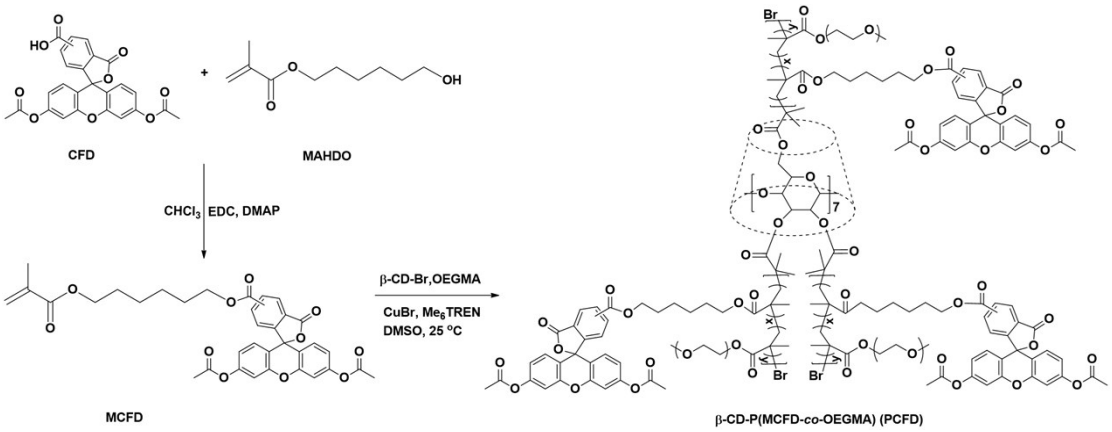

Figure S1.Synthesis of β-CD-P(MCFD-*co*-OEGMA) (PCFD) copolymer.

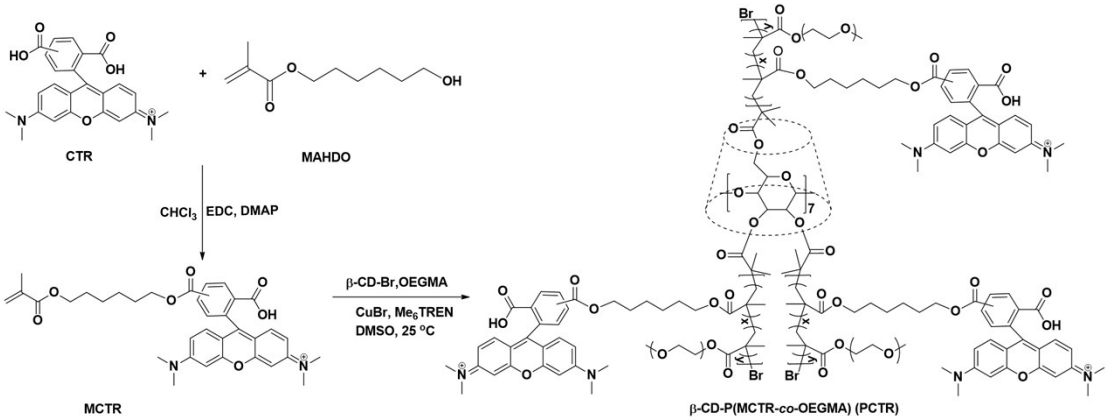

Figure S2.Synthesis of β-CD-P(MCTR-*co*-OEGMA) (PCTR) copolymer.

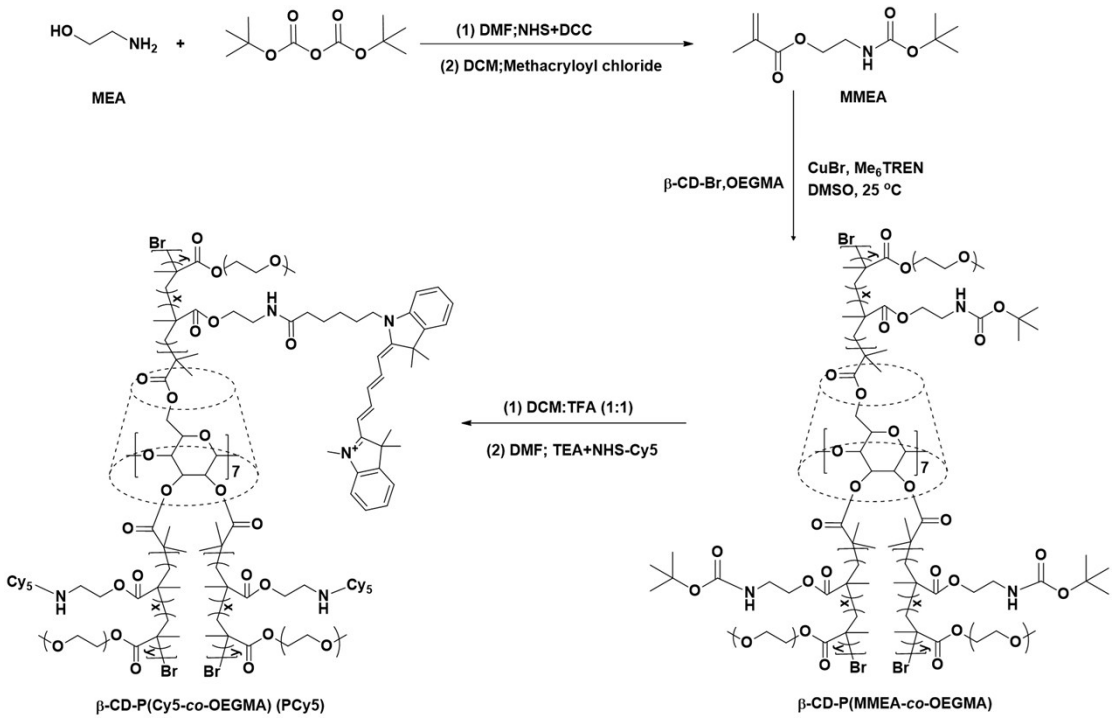

Figure S3.Synthesis of β-CD-P(Cy5-*co*-OEGMA) (PCy5) copolymer.

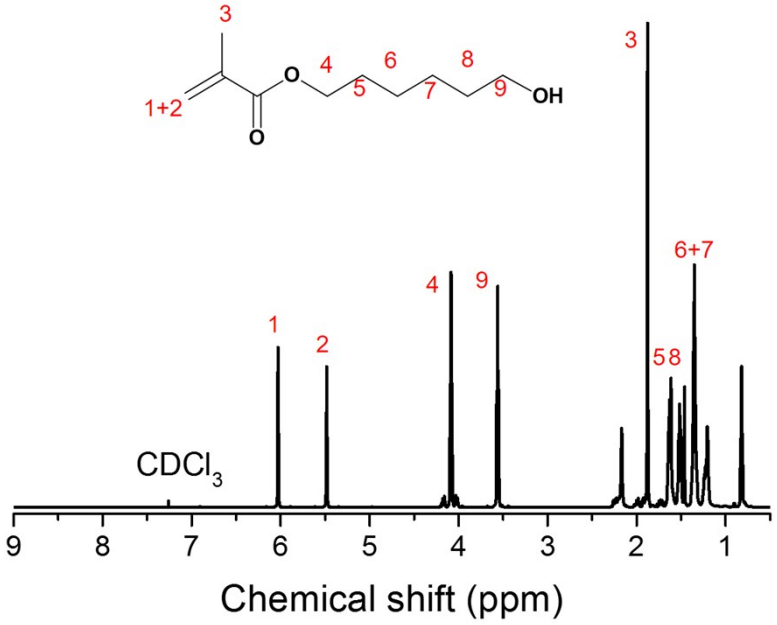

Figure S4. [1]H NMR spectrum of MAHDO.

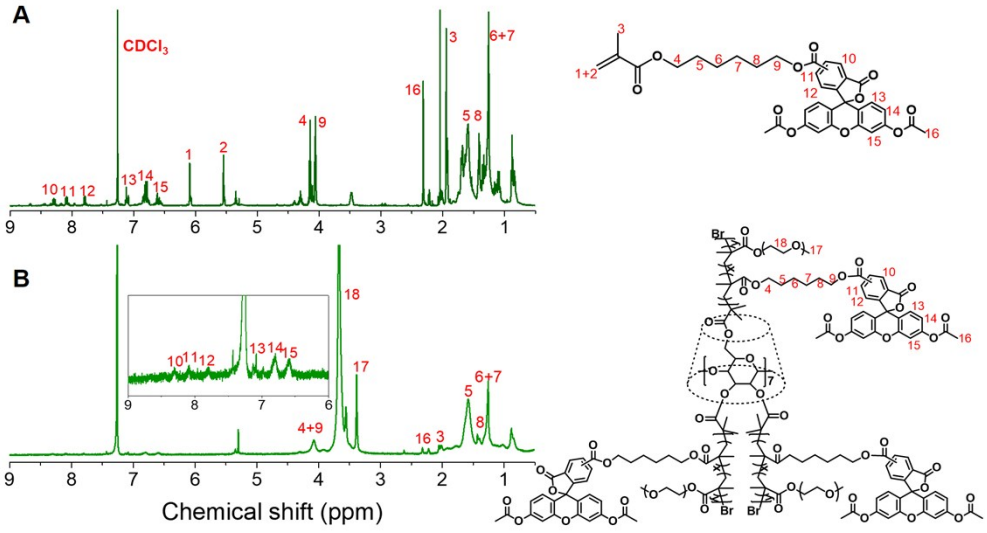

Figure S5. [1]H NMR spectra of MCFD (A) and PCFD (B).

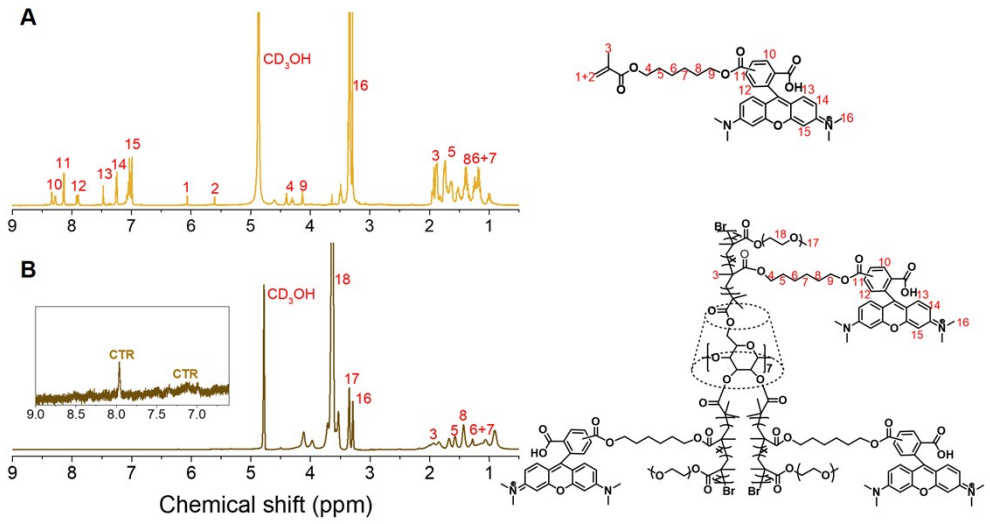

Figure S6. ^{1}H NMR spectra of MCTR (A) and PCTR (B).

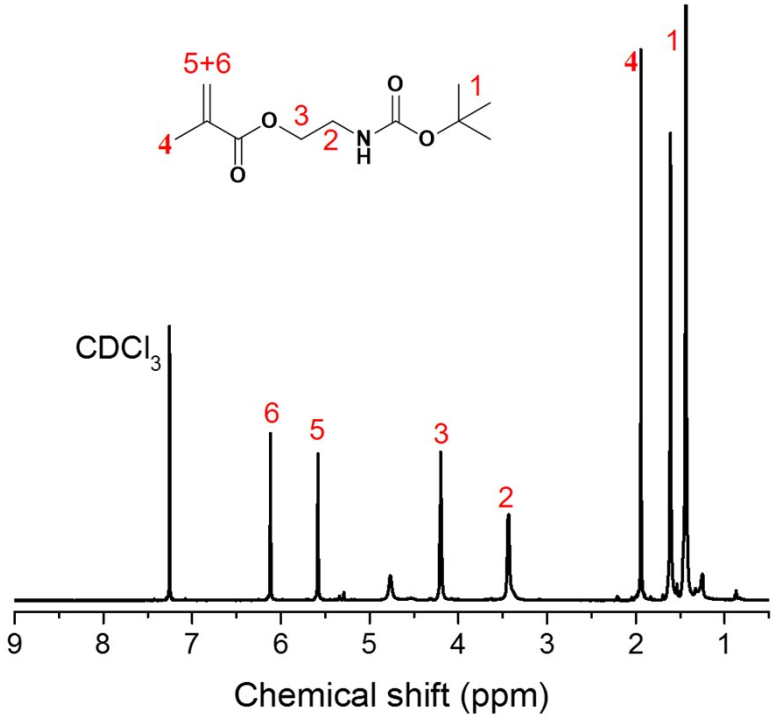

Figure S7. ^{1}H NMR spectrum of MMEA.

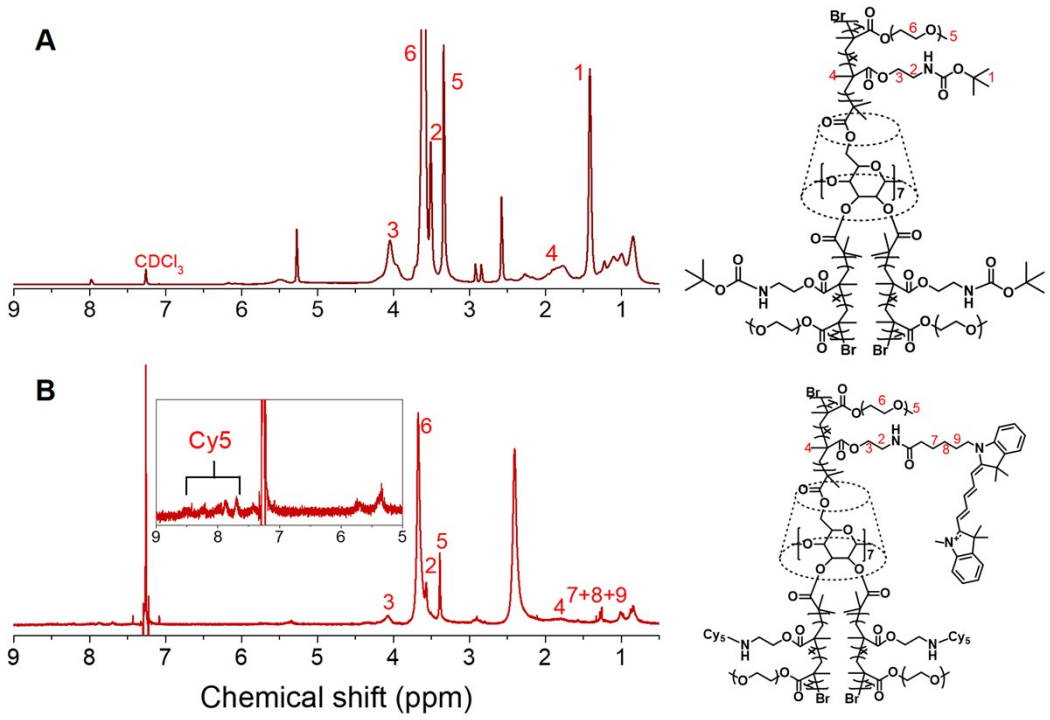

Figure S8. ^{1}H NMR spectra of β-CD-P(MMEA-*co*-OEGMA) (A) and (B)PCy5.

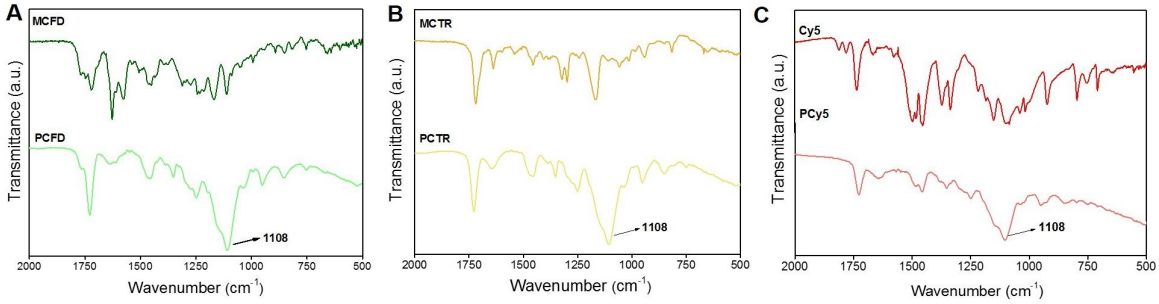

Figure S9. The FT-IR spectra of MCFD monomer and PCFD polymer (A), MCTR monomer and PCTR polymer (B), Cy5 and PCy5 polymer (C).

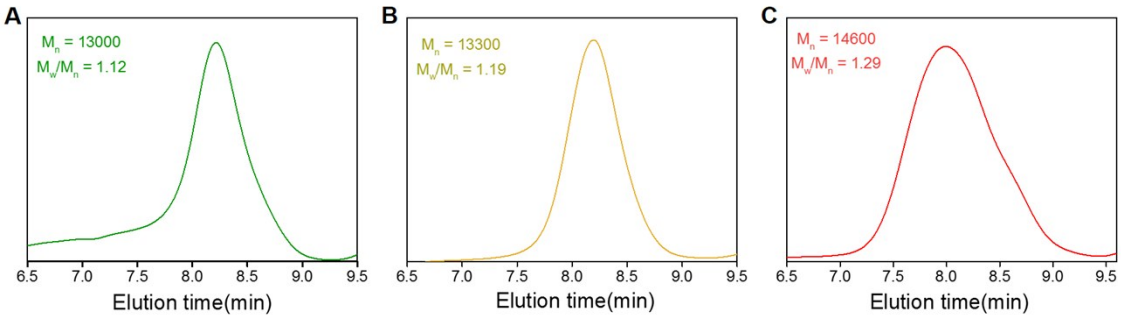

Figure S10. GPC traces of PCFD (A), PCTR (B) and β-CD-P (MMEA-*co*-OEGMA) (C).

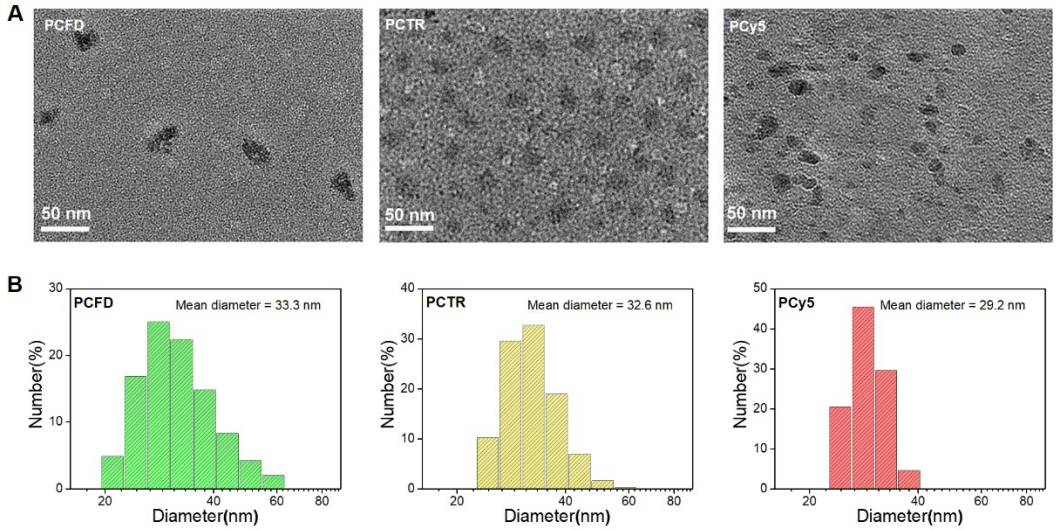

Figure S11. The TEM images (A) of PCFD, PCTR and PCy5 UMs and DLS results (B) of PCFD, PCTR and β-CD-P(MMEA-*co*-OEGMA) UMs in aqueous medium.

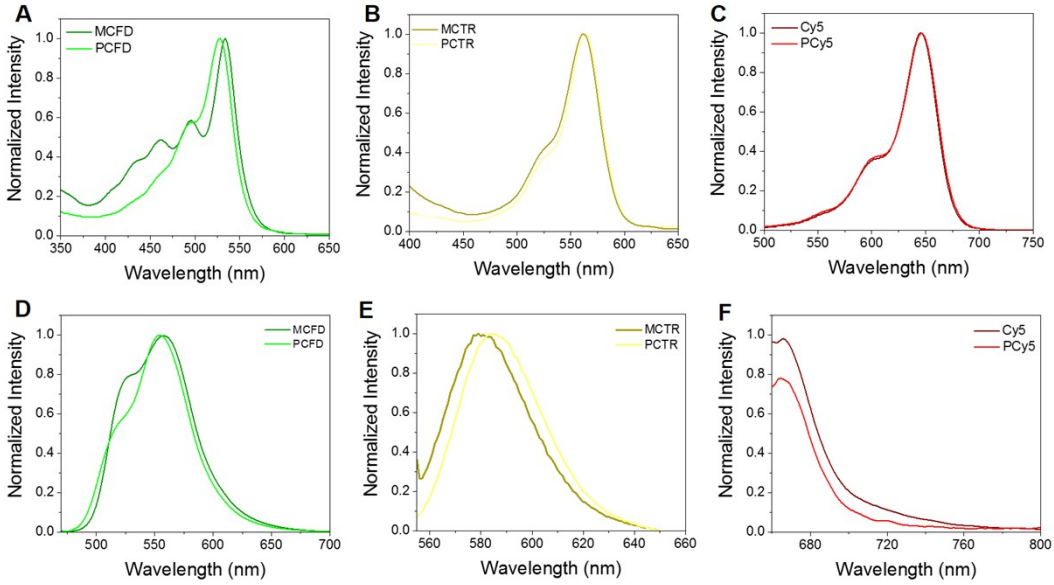

Figure S12. UV-vis spectra of MCFD and PCFD (A), MCTR and PCTR(B) , Cy5 and PCy5(C) in DMF solution; fluorescence emission spectra of MCFD and PCFD (D), MCTR and PCTR(E), Cy5 and PCy5 (F) in DMF solution.

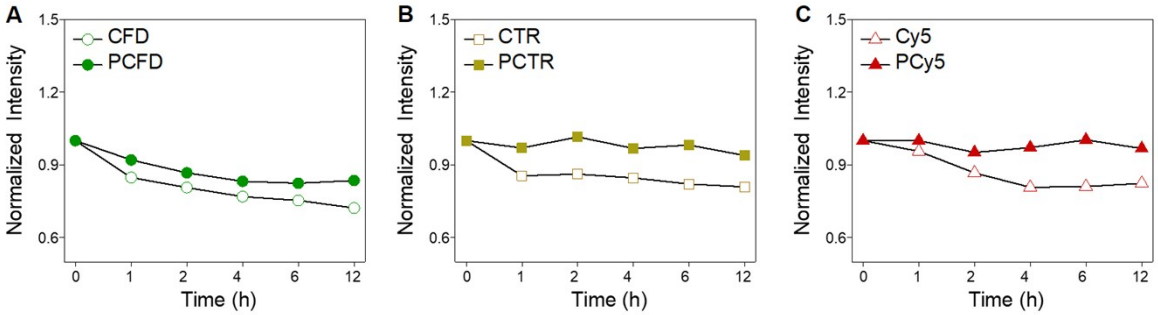

Figure S13. Normalized fluorescence intensity over time during photostability study of CFD or PCFD (A), CTR or PCTR (B) and Cy5 or PCy5 (C) UMs.

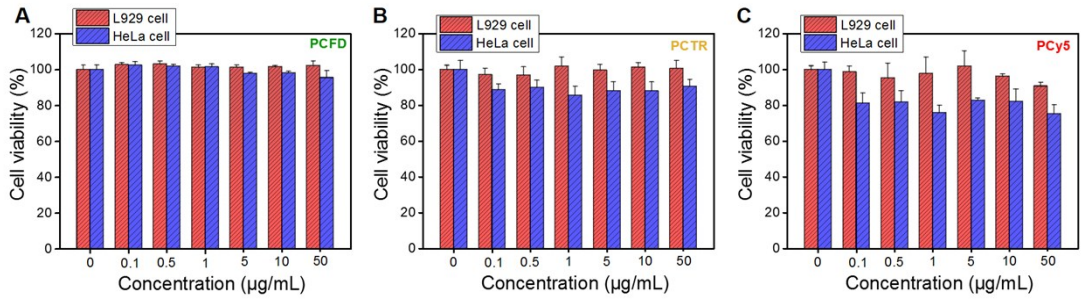

Figure S14. Cell viability of HeLa cells and L-929 cells incubated with PCFD (A), PCTR (B) and PCy5 (C) UMs for 72 h.

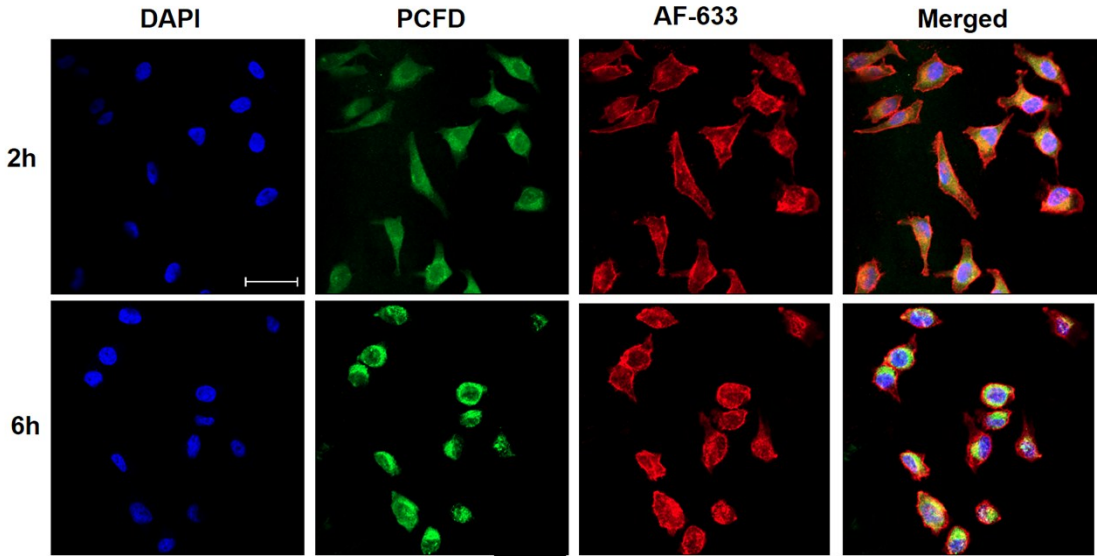

Figure S15. CLSM images of HeLa cells after incubated with PCFD UMs for 2 and 6 h. The fluorescence of PCFD, AF-633 and DAPI were labeled green, red and blue. Scale bars: 50μm.

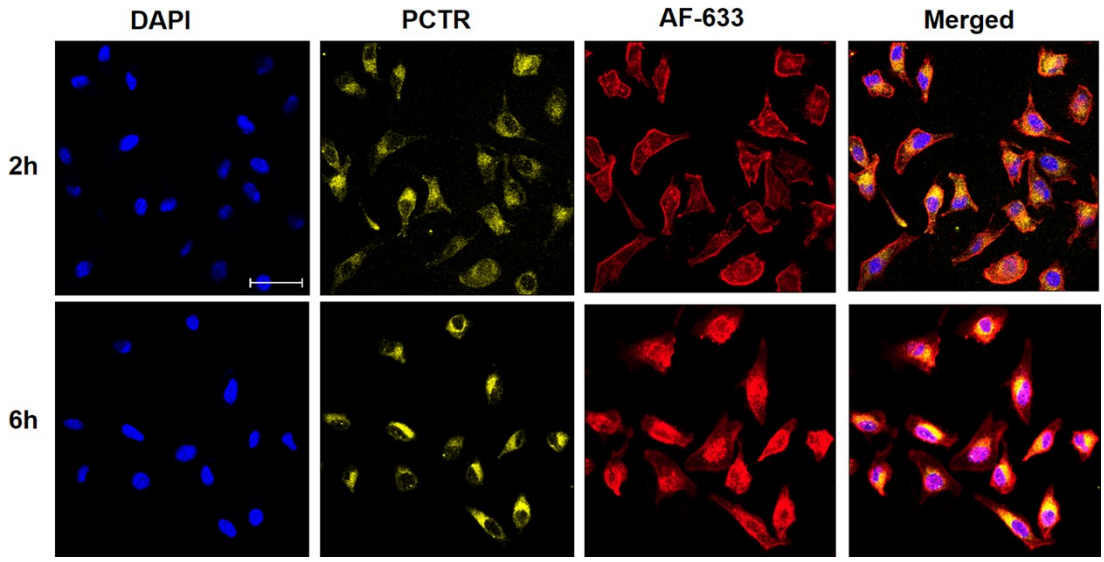

Figure S16. CLSM images of HeLa cells after incubated with PCTR UMs for 2 and 6 h. The fluorescence of PCTR, AF-633 and DAPI were labeled yellow, red and blue. Scale bars: 50μm.

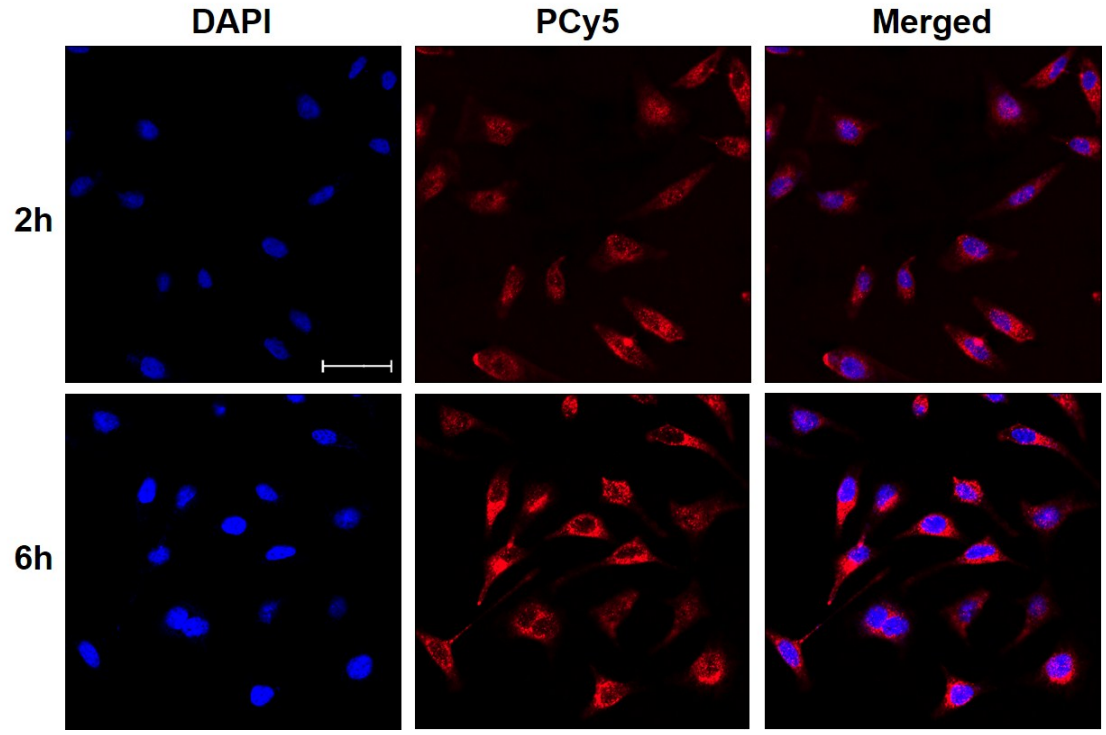

Figure S17. CLSM images of HeLa cells after incubated with PCy5 UMs for 2 and 6 h. The fluorescence of PCy5 and DAPI were labeled red and blue. Scale bars: 50μm

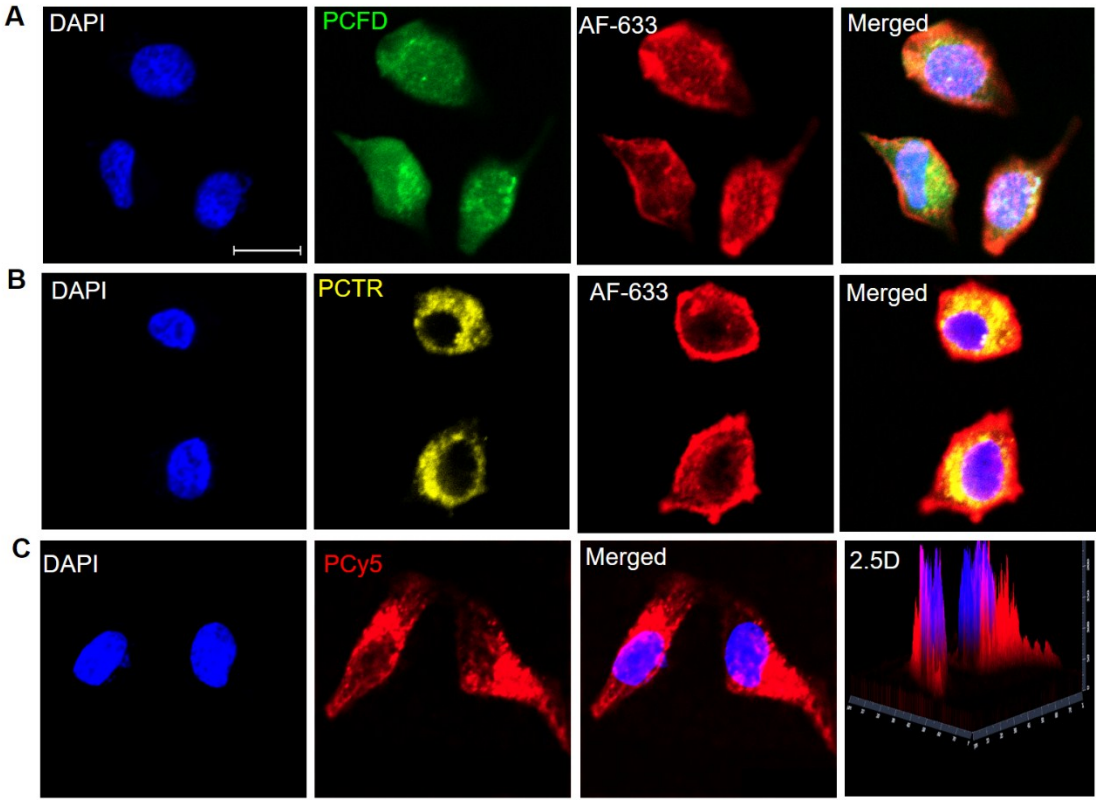

Figure S18. CLSM images of HeLa cells after incubated with PCFD (A), PCTR (B) and PCy5(C) UMs for 6 h. The fluorescence of PCFD, PCTR，PCy5，AF-633 and DAPI were labeled green, yellow, red, red and blue, respectively. Scale bars: 25μm.

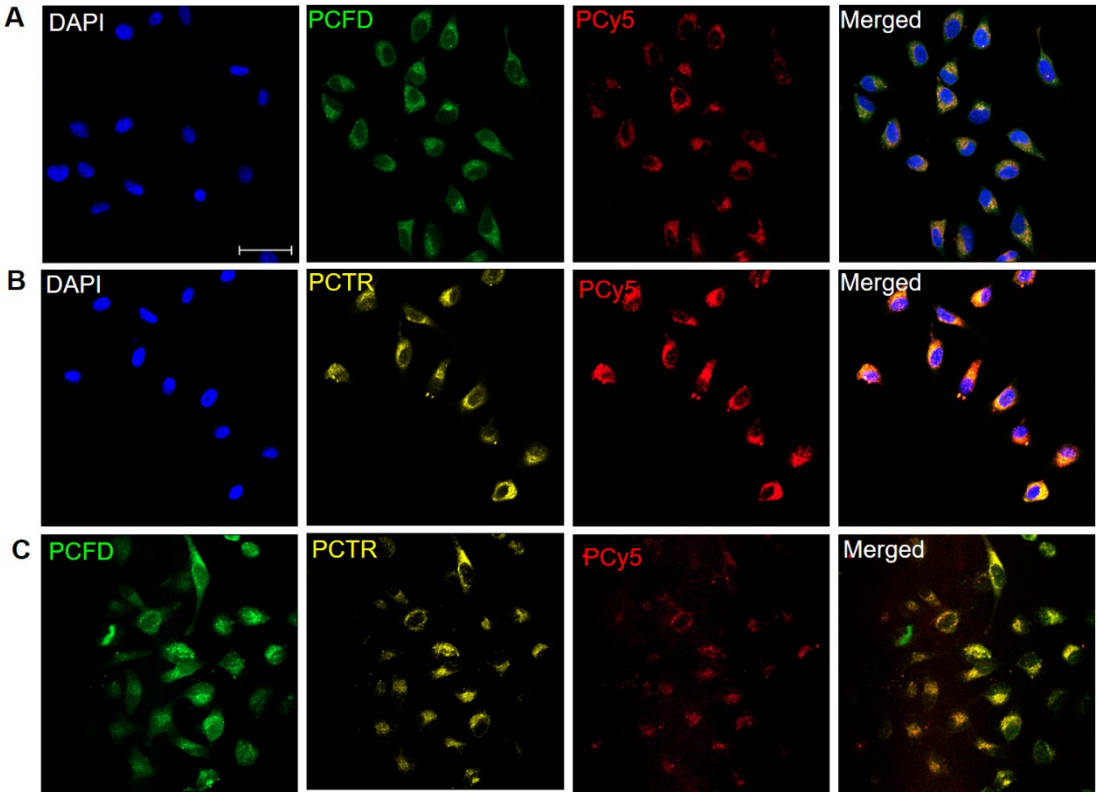

Figure S19. CLSM images of HeLa cells after incubated with mixed UMs of PCFD/PCy5 (A), PCTR/PCy5 (B), PCFD/PCTR/PCy5 (C) for 6 h. The fluorescence of PCFD, PCTR, PCy5 and DAPI were labeled green, yellow, red and blue, respectively. Scale bars: 50μm.

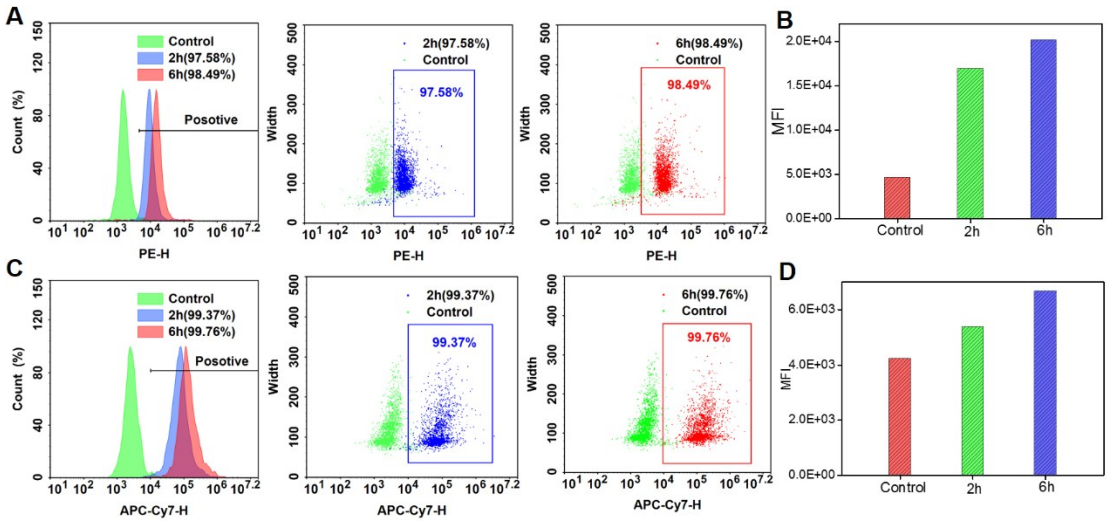

Figure S20. The flow cytometry analysis of HeLa cells after treatment with PCFD (A and B) and PCy5 (C and D) UMs for 2 and 6 h.

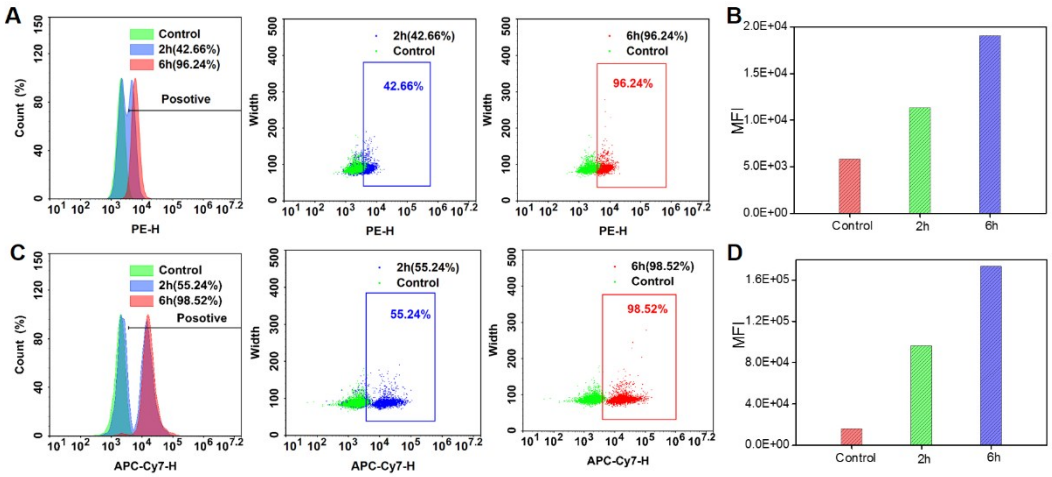

Figure S21. The flow cytometry analysis of HeLa cells after treatment with PCFD/PCy5 mixed UMs for 2h (A and B) and 6h (C and D).

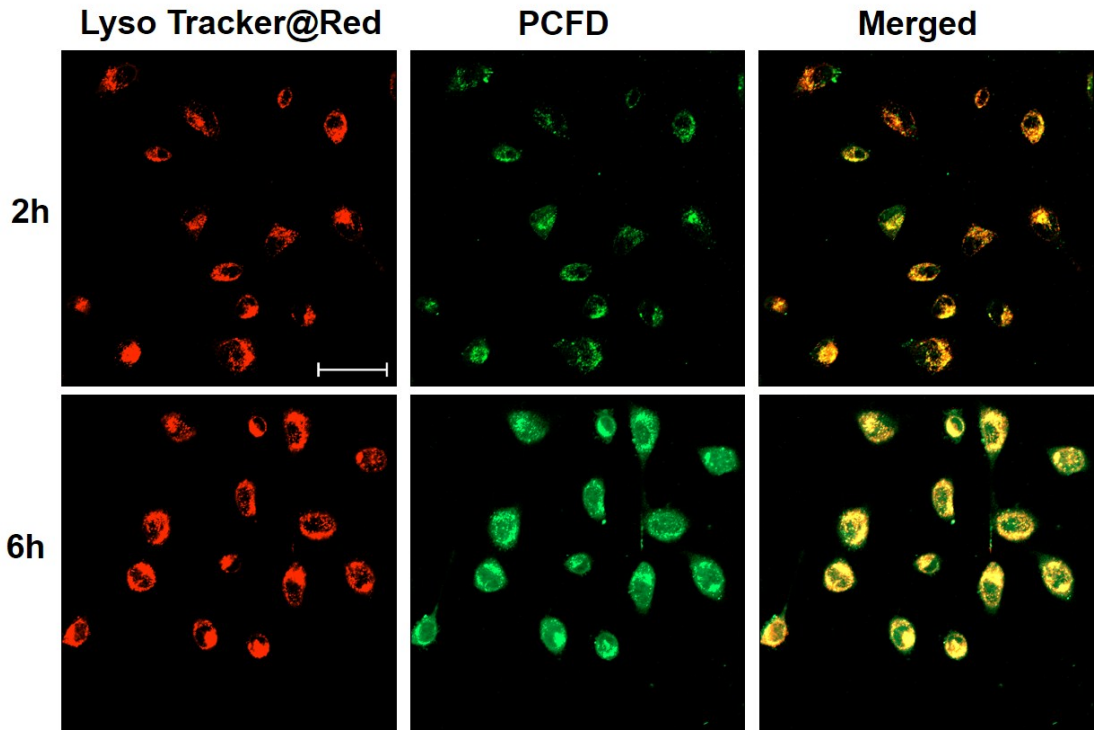

Figure S22. CLSM images of HeLa cells after incubated with PCFD UMs for 2 and 6 h. The fluorescence of PCFD and Lyso Tracker@Red were labeled green and red, respectively. Scale bars: 50μm.

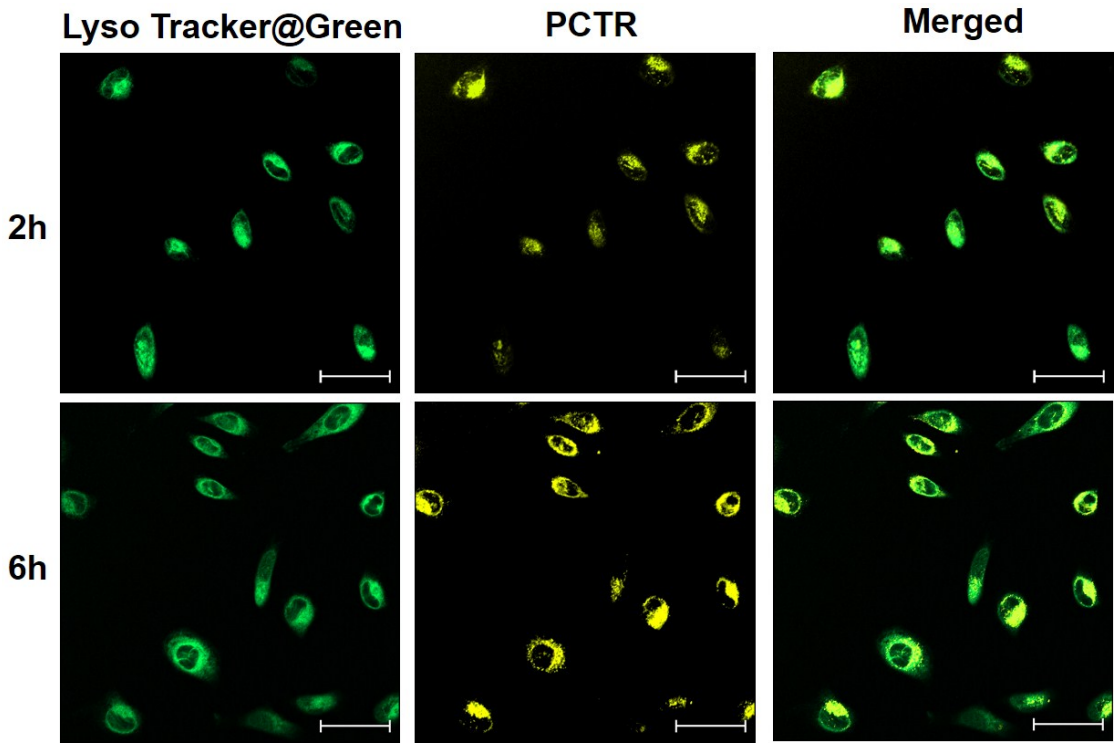

Figure S23. CLSM images of HeLa cells after incubated with PCTR UMs for 2 and 6 h. The fluorescence of PCTR and Lyso Tracker@Green were labeled yellow and green, respectively. Scale bars: 50μm.

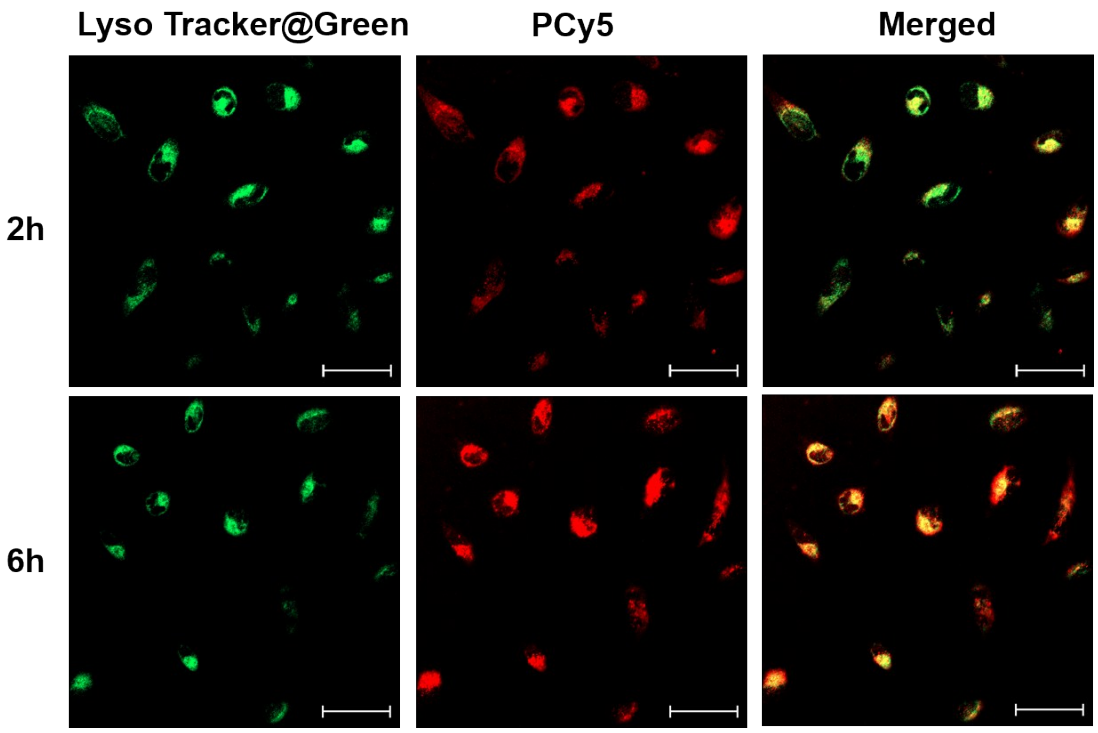

Figure S24. CLSM images of HeLa cells after incubated with PCy5 UMs for 2 and 6 h. The fluorescence of PCy5 and Lyso Tracker@Green were labeled red and green, respectively. Scale bars: 50μm.

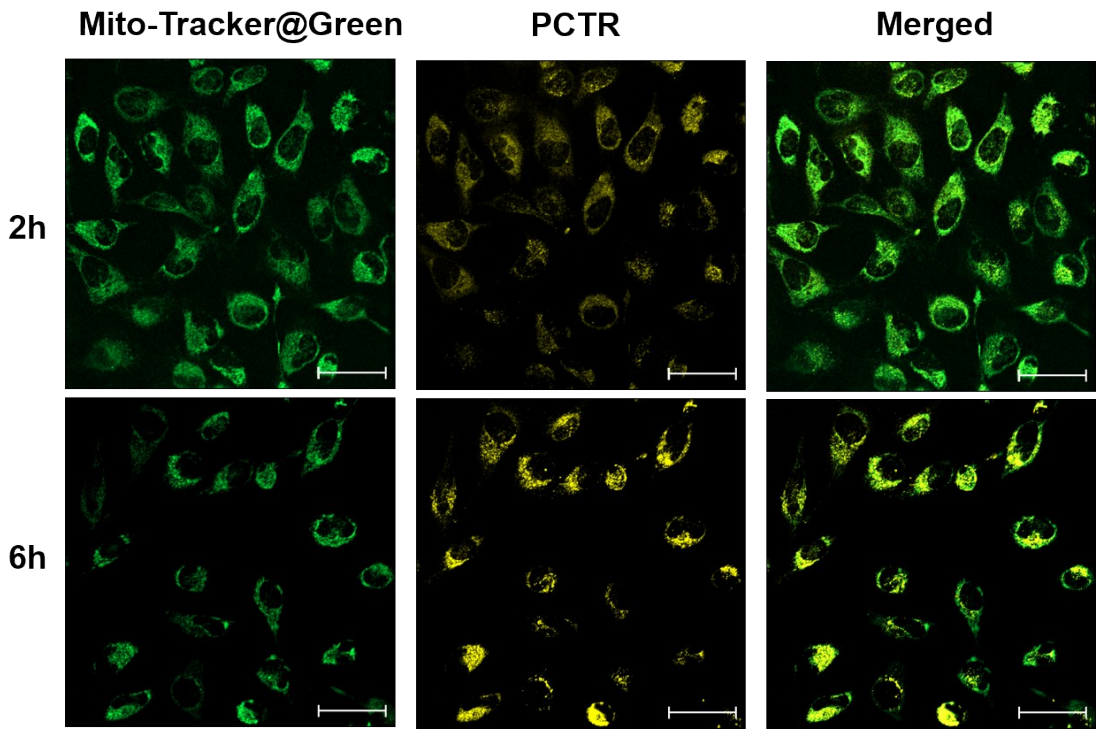

Figure S25. CLSM images of HeLa cells after incubated with PCTR UMs for 2 and 6 h. The fluorescence of PCTR and Mito-Tracker@Green were labeled yellow and green, respectively. Scale bars: 50μm.

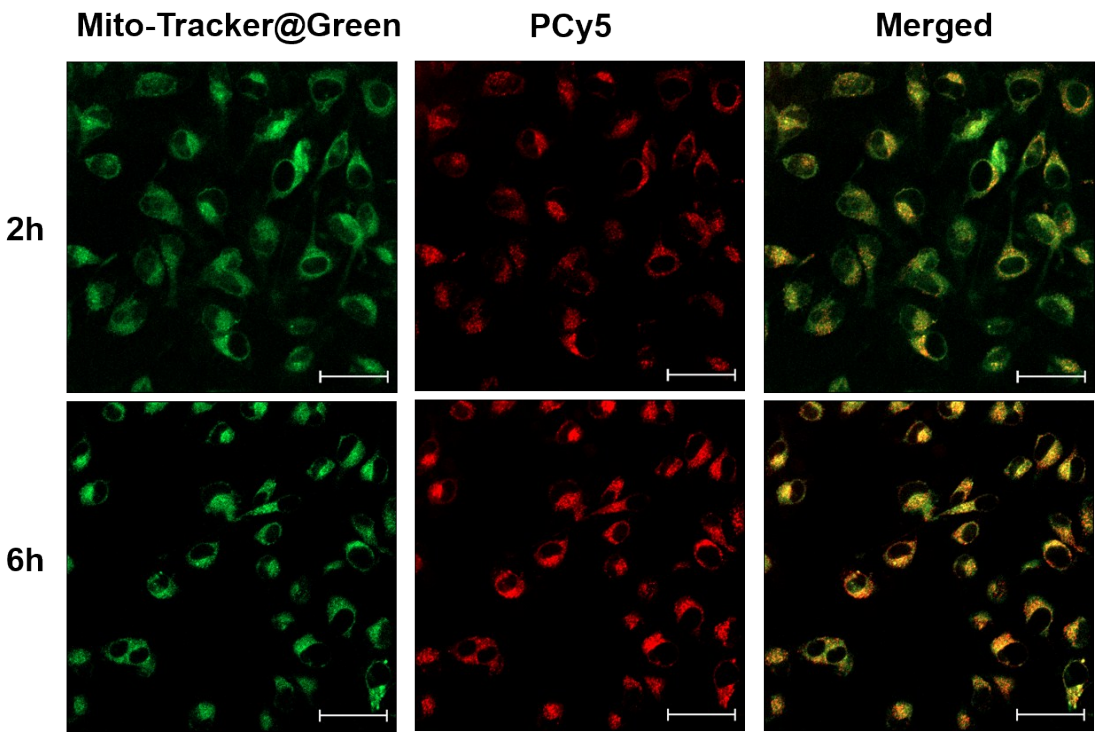

Figure S26. CLSM images of HeLa cells after incubated with PCy5 UMs for 2 and 6 h. The fluorescence of PCy5 and Mito-Tracker@Green were labeled red and green, respectively. Scale bars: 50μm.

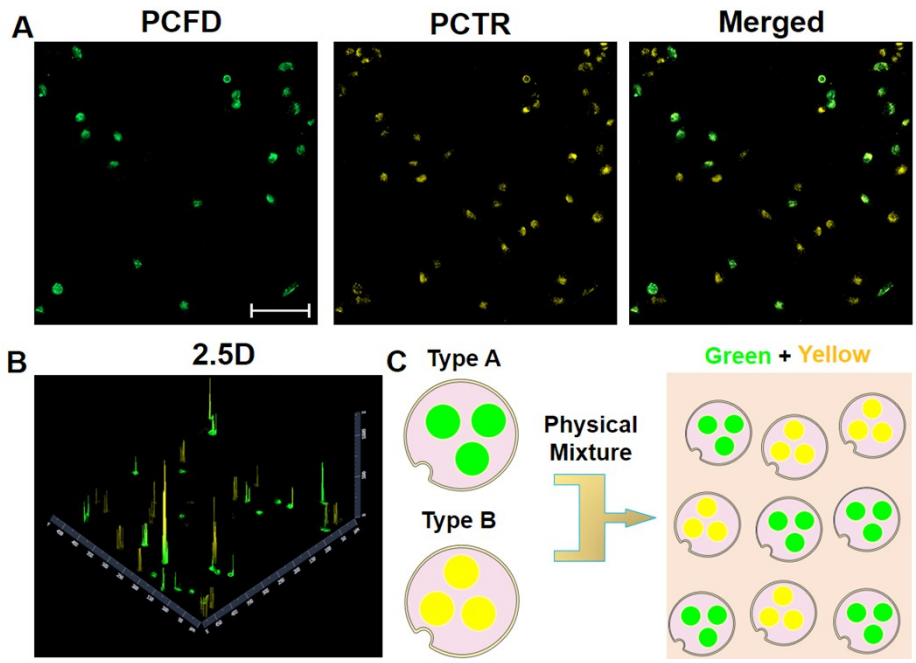

Figure.S27 (A) CLSM images of HeLa cells by physical mixture after incubated with PCFD and PCTR UMs for 6 h. (B) The 2.5-dimensional (2.5 D) image of merged photos. (C) The mechanism representative chart was attached. The fluorescence of CFD and CTR were labeled green and yellow. Scale bars: 50μm

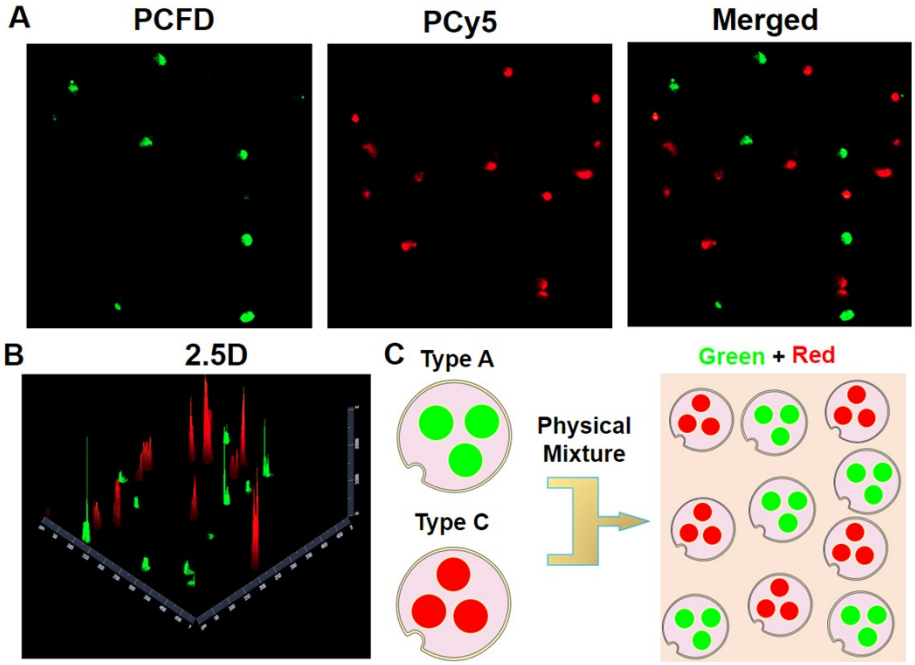

Figure.S28 (A) CLSM images of HeLa cells by physical mixture after incubated with PCFD and PCy5 UMs for 6 h. (B) The 2.5-dimensional (2.5 D) image of merged photos. (C) The mechanism representative chart was attached. The fluorescence of PCFD and PCy5 were labeled green and red. Scale bars: 50μm

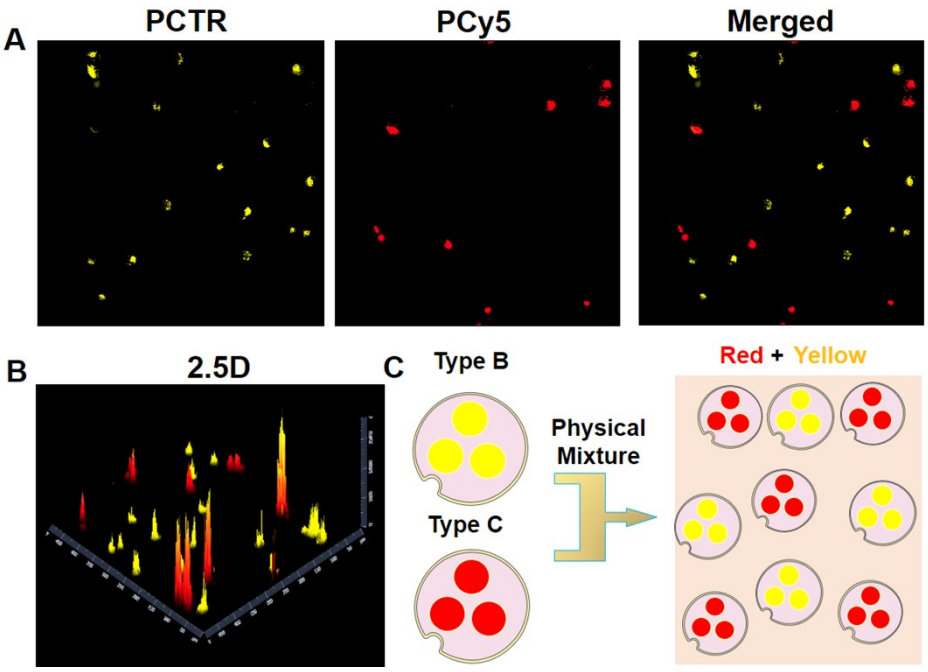

Figure.S29 (A) CLSM images of HeLa cells by physical mixture after incubated with PCTR and PCy5 UMs for 6 h. (B) The 2.5-dimensional (2.5 D) image of merged photos. (C) The mechanism representative chart was attached. The fluorescence of PCTR and PCy5 were labeled yellow and red. Scale bars: 50μm

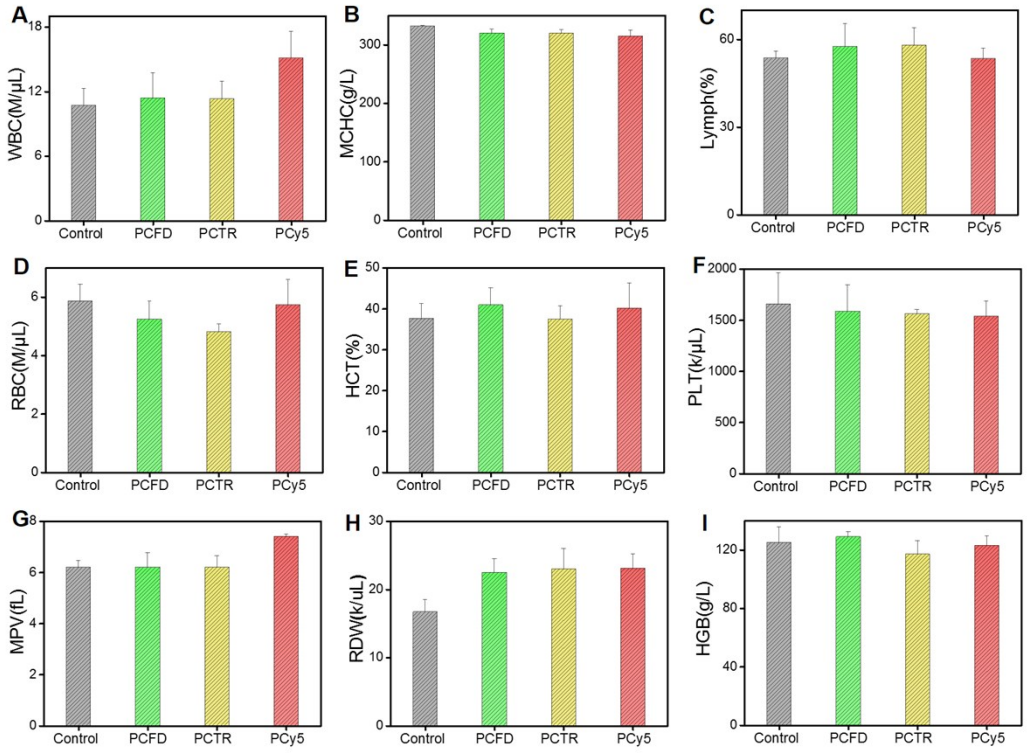

Figure S30. SD mice were intravenously injected with PBS, PCFD, PCTR and PCy5 UMs for 7 days, respectively. Blood test parameters: WBC (A), MCHC (B), Lymph (C), RBC (D), HCT (E), PLT (F), MPV (G), RDW (H), and HGB (I). Data are represented as mean ± SD (n =3).

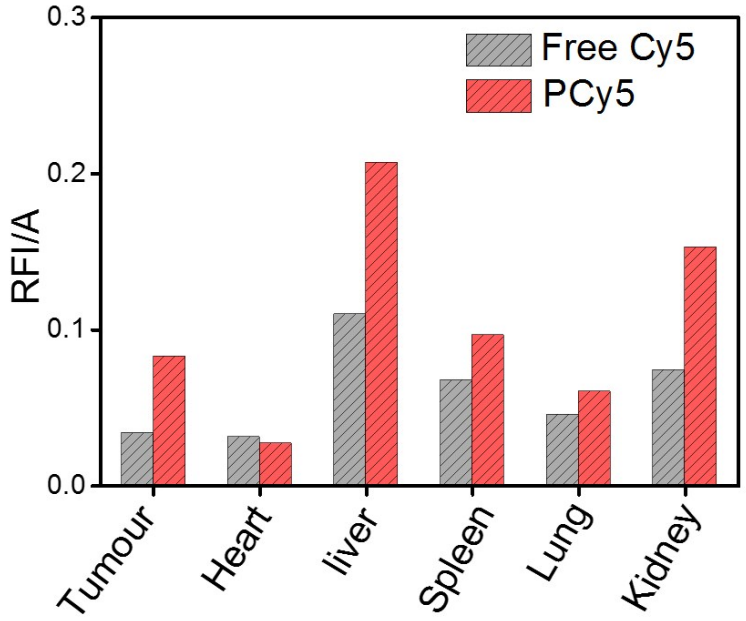

Figure S31. The distribution profiles of free Cy5 and PCy5 UMs in excised organs based on the relative fluorescent intensity (RFI)/Area (RFI/A) at 24 h post-injection.

Table S1. Quantum yield (Φ) of free dyes and fluorescent unimolecular micelles (FUMs)

Sample	CFD (Φ%)	CTR (Φ%)	Cy5 (Φ%)
free Dye	95[3]	12[3]	27[4]
FUMs	100	81.46	0.47

2. References

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