Supporting information for:

Hydrogen-Bonded Supramolecular Polymer Micelles with Photothermal-Responsive Carmofur Release and Combined Chemo-Photothermal Therapy

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Materials

Poly(vinyl alcohol) (PVA, M_w = 13-23 KDa) and IR780 iodide were purchased from Sigma-Aldrich (USA). Carmofur (HCFU) was purchased from TCI (Japan). Phosphate buffer solution (PBS, 0.01M), DMEM (high glucose) and fetal bovine serum (FBS) were obtained from Gibco (USA). Trypsin-EDTA solution with phenol red, penicillin-streptomycin solution and Paraformaldehyde (4%) were purchased from Biosharp (China). All the chemicals were used without further purification.

Characterization

^{1}H NMR spectra were performed on a Bruker AscendTM 600 MHz spectrometer using $CDCl_3$ as solvent.

Transmission electron microscopy (TEM) investigation was conducted with an HT7700 electron microscope with a CCD camera operating at an acceleration voltage of 120 kV. The samples (~5 μL) were drop-casted onto a Cu grid coated with a carbon film and air-dried before the measurement without staining.

Dynamic light scattering (DLS) measurement was conducted on a Malvern Nano-ZS90 using OmniSIZE software.

UV-visible (UV-vis) spectroscopic studies were performed on LAMBDA 45, Perkins Ehmer to calculate the loading content of HCFU and IR780 of supramolecular polymeric micelles.

Thermal Fotric 226s infrared camera was used to monitor the temperature change during the process of photothermal conversion by the micelles.

Confocal laser scanning microscopy (CLSM) studies were performed on Leica TCS SP5.

Experimental Section

Mathematical model chosen to determine K_a: Data related to the concentration-dependence of the chemical shift of HCFU N*H* proton for a mixture of HCFU and POEGEA-*b*-P(DAPA-*co*-TFEA)-*b*-POEGEA were fitted according to the following equation[1] to provide K_a:

$$\delta_{mixt} = \delta_{HCFU} + \frac{(\delta_\infty - \delta_{HCFU})(\left([HCFU] + [DAP] + \frac{1}{K_a}\right) - (([HCFU] + [DAP] + \frac{1}{K_a})^2 - 4[HCFU][DAP])^{1/2})}{2[HCFU]}$$

where the experimental parameters are: [HCFU] and [DAP], the respective molar concentrations of HCFU and POEGEA-*b*-P(DAPA-*co*-TFEA)-*b*-POEGEA; δ_{mixt}, the measured N*H* chemical shift for HCFU and POEGEA-*b*-P(DAPA-*co*-TFEA)-*b*-POEGEA mixture; δ_{HCFU}, the measured N*H* chemical shift. The fitted parameters are: K_a, the association constant and δ_∞, the N*H* chemical shift of the fully associated system.

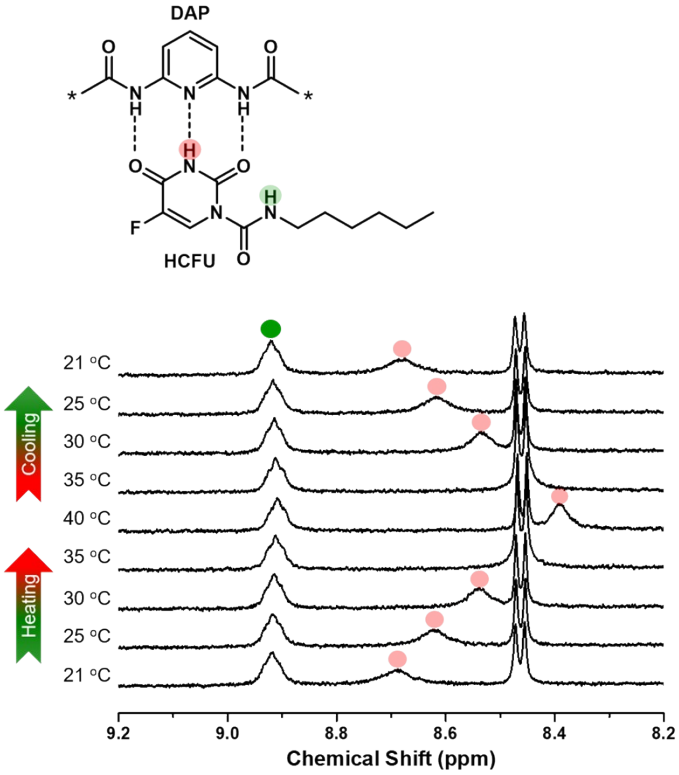

Fig. S1: Temperature-dependence of the chemical shift of HCFU N*H* proton (red spots) for a mixture of POEGEA-*b*-P(DAPA-*co*-TFEA)-*b*-POEGEA and HCFU ([POEGEA-*b*-P(DAPA-*co*-TFEA)-*b*-POEGEA] = [HCFU] = 3 mM).

Preparation of the polymeric micelles via the emulsion-solvent evaporation method

The polymeric micelles generated from POEGEA-*b*-P(DAPA-*co*-TFEA)-*b*-POEGEA or POEGEA-*b*-PTFEA-*b*-POEGEA were prepared by using the emulsification method,[2] followed by the solvent evaporation at 30 °C for 24 h. POEGEA-*b*-P(DAPA-*co*-TFEA)-*b*-POEGEA or POEGEA-*b*-PTFEA-*b*-POEGEA (15 mg) was dissolved in $CHCl_3$ (5 mL), then the obtained solution was emulsified with PVA aqueous solution (3 mg/mL, 10 mL) by ultrasonication (total time: 5 min) in a vial placed in the ice-water bath. $CHCl_3$ was then allowed to fully evaporate in an open vial at 30 °C for 24 h, eventually leading to the solidification of polymers. The particles were thus obtained through centrifugation of the resulting suspension to eliminate the redundant PVA, and finally the obtained particles were dispersed in distilled water and subjected to TEM and DLS investigations.

Preparation of HCFU&IR780-loaded (supramolecular) polymeric micelles

The supramolecular polymeric micelles generated from POEGEA-*b*-P(DAPA-*co*-TFEA)-*b*-POEGEA@HCFU/IR780, or POEGEA-*b*-PTFEA-*b*-POEGEA@HCFU/IR780 were prepared using the same emulsification method under the same experimental conditions. POEGEA-*b*-P(DAPA-*co*-TFEA)-*b*-POEGEA (15 mg), or POEGEA-*b*-PTFEA-*b*-POEGEA (15 mg), was mixed with chemotherapy drug HCFU (6 mg) and photothermal agent IR780 (6 mg), then dissolved in $CHCl_3$ (5 mL) followed by the stirring overnight to complete the H-bonding association between polymeric carrier and HCFU. Then the obtained solution was emulsified with PVA aqueous solution (3 mg/mL, 10 mL) by ultrasonication (total time: 5 min) in a vial placed in the ice-water bath. $CHCl_3$ was then allowed to fully evaporate in an open vial at 30

°C for 24 h, eventually leading to the solidification of polymers. The particles were thus obtained through centrifugation of the resulting suspension to eliminate the redundant PVA, subsequently the free HCFU and IR780 were removed completely by dialysis (MWCO=14000 Da) against $CHCl_3$, and finally the obtained POEGEA-*b*-P(DAPA-*co*-TFEA)-*b*-POEGEA@HCFU/IR780 and POEGEA-*b*-PTFEA-*b*-POEGEA@HCFU/IR780 micelles were dispersed in distilled water and subjected to critical micelle concentration (CMC) measurements, TEM and DLS investigation.

We use pyrene as the hydrophobic fluorescent probe to measure CMC values.[3] Briefly, POEGEA-*b*-P(DAPA-*co*-TFEA)-*b*-POEGEA micelles and POEGEA-*b*-PTFEA-*b*-POEGEA micelles solution was diluted to various concentrations ranging from 50 mg/L to 0.1 mg/L. After that, pyrene probe in acetone solution with final concentration of 6×10^{-7} M was added into the micelles. The mixture was stirred in the dark for 2 h and completely evaporating the acetone under reduced pressure, the fluorescence emission spectra were adjusted from 360 to 440 nm at an excitation wavelength of 335 nm. The CMC was determined as the cross-point when the intensity ratio of I_{383}/I_{372} was plotted at low and high concentration regions.

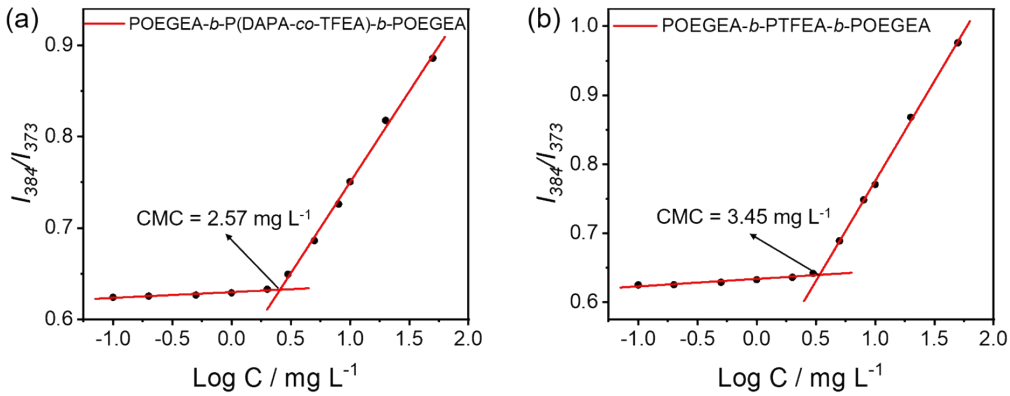

Fig. S2: Plots of the I_{384}/I_{373} ratio (from pyrene excitation spectra at λ_{ex} = 335 nm) versus Log C of POEGEA-*b*-P(DAPA-*co*-TFEA)-*b*-POEGEA (a) and POEGEA-*b*-PTFEA-*b*-POEGEA (b).

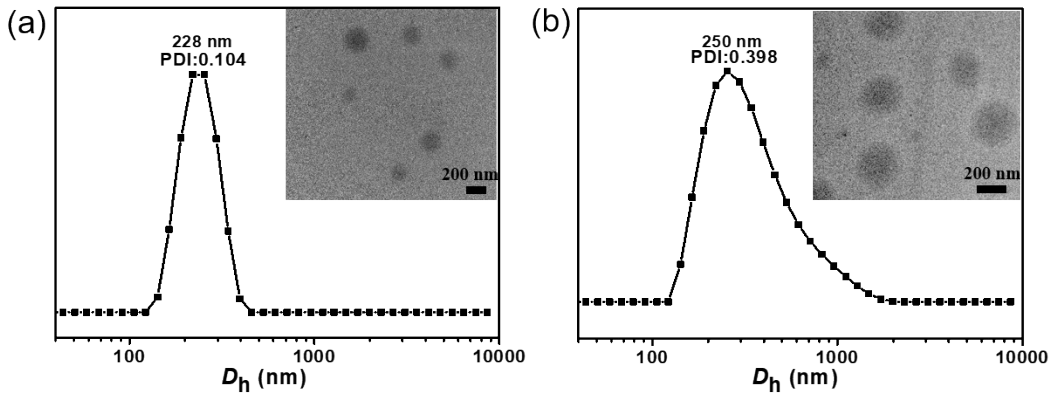

Fig. S3: DLS results and inset TEM images of POEGEA-*b*-PTFEA-*b*-POEGEA (bottom row) without (a) and with (b) HCFU/IR780 loading.

Measurement of HCFU and IR780 loading contents

POEGEA-*b*-P(DAPA-*co*-TFEA)-*b*-POEGEA@HCFU/IR780, and the control micelles POEGEA-*b*-PTFEA-*b*-POEGEA@HCFU/IR780 are dissolved in PBS buffer solution (pH = 7.0/6.0/5.0), along with vigorous shaking for 30 min. The absorbance is assessed by UV-Vis spectrophotometry at a wavelength of 260 nm for HCFU, and 774 nm for IR780, respectively. Drug loading content (DLC, %) and drug loading efficiency (DLE, %) of HCFU or IR780 in POEGEA-*b*-P(DAPA-*co*-TFEA)-*b*-POEGEA@HCFU/IR780 and POEGEA-*b*-PTFEA-*b*-POEGEA@HCFU/IR780 were calculated according to following equations, respectively. DLC and DLE (**Table S1**) of the drug-loaded micelles were calculated according to following equations, respectively:

$$DLC\ (\%) = \frac{The\ mass\ of\ drug\ in\ the\ NP}{The\ mass\ of\ the\ NP} \times 100\%$$

$$DLE\ (\%) = \frac{The\ mass\ of\ drug\ in\ the\ NP}{The\ total\ mass\ of\ the\ added\ drug} \times 100\%$$

Table S1: DLC and DLE of HCFU and IR780 of POEGEA-*b*-P(DAPA-*co*-TFEA)-*b*-POEGEA and POEGEA-*b*-PTFEA-*b*-POEGEA.

Sample	DLC/DLE of HCFU (%/%)	DLC/DLE of IR780 (%/%)
POEGEA-*b*-P(DAPA-*co*-TFEA)-*b*-POEGEA @HCFU/IR780	15.6 ± 1.5/40.4 ± 1.9	5.8 ± 2.4/38.7 ± 1.5
POEGEA-*b*-PTFEA-*b*-POEGEA@HCFU/IR780	10.8 ± 1.6/28.0 ± 2.1	4.5 ± 2.1/29.6 ± 1.4

Photothermal conversion properties

The photothermal conversion performance of POEGEA-*b*-P(DAPA-*co*-TFEA)-*b*-POEGEA@HCFU/IR780 micelles were measured at different concentrations or powers under 808 nm laser irradiation. The micelles were dispersed in water to form dispersions with concentrations of 0.55, 1.1, 1.65 mg/mL. Temperature of the dispersions was monitored in real time under the laser irradiation of 1 W/cm^2 power. Subsequently, a dispersion with a concentration of 1.1 mg/mL was selected to monitor the temperature in real time under 808 nm laser irradiation with powers of 0.6, 1, 1.5, and 2 W/cm^2.

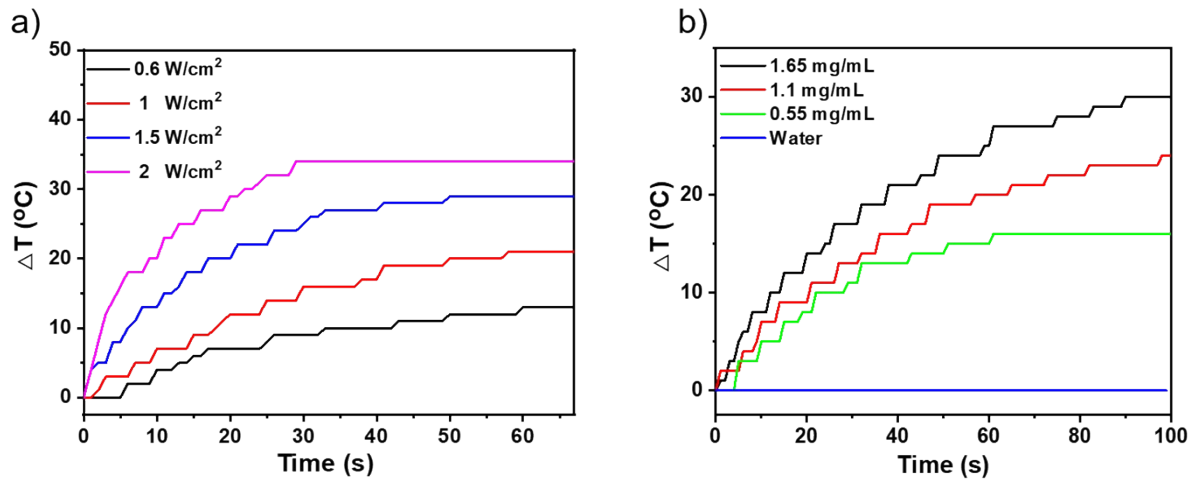

Fig. S4: Laser power-dependent (a) and concentration-dependent (b) photothermal conversion of POEGEA-*b*-P(DAPA-*co*-TFEA)-*b*-POEGEA@HCFU/IR780 micelles.

In vitro HCFU release profiles of micelles

0.01 M PBS was selected to simulate the drug release environment in the body. Due to the sensitivity of hydrogen bonds to temperature and pH, the release profiles of HCFU from POEGEA-*b*-P(DAPA-*co*-TFEA)-*b*-POEGEA@HCFU/IR780 micelles were studied separately in PBS with pH 7.4, 6.5 and 5.5. And 808 nm laser irradiate was given to studied its effect on the release of HCFU.

The release profiles of HCFU were determined through a dialysis method. Quantified micelles were dispersed in PBS with pH 7.4, 6.5, or 5.5, respectively. Then, the dispersions were transferred into three dialysis tubes (MWCO=14000 Da) separately and dialyzed with PBS (pH = 7.4, 6.5, or 5.5) at 37 °C in the shaker. At different time points, external solution of PBS (400 μL) was taken out to determine the concentration of HCFU by UV-Vis spectrophotometer and was supplemented with an equal volume of fresh PBS (pH 7.4, 6.5, 5.5). The NIR-triggered HCFU drug release profiles from POEGEA-*b*-P(DAPA-*co*-TFEA)-*b*-

POEGEA@HCFU/IR780 micelles was measured by using a similar approach of no-irradiation. NIR laser irradiation (1 W/cm^2, 3 min; 1 W/cm^2, 5 min) was applied at drug releasing time of 5 h and 26 h, respectively, and then the dispersions were placed in the shaker again.

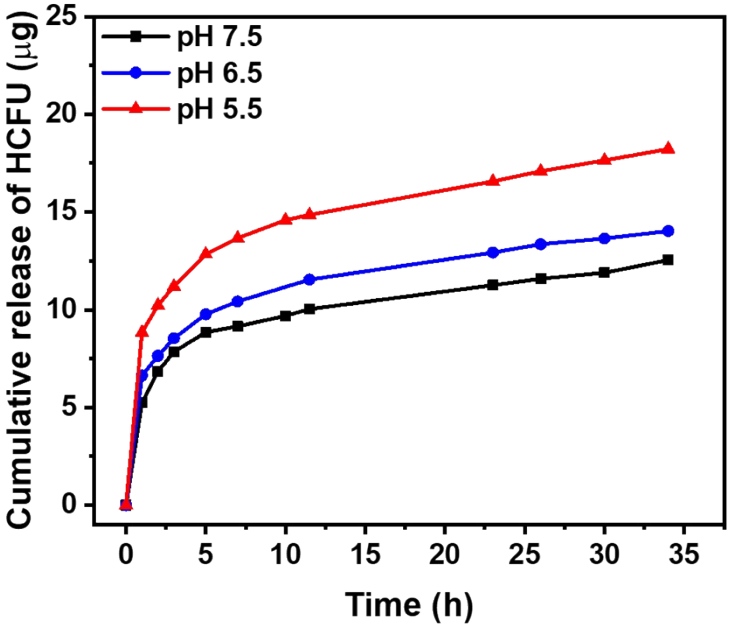

Fig. S5: In vitro drug release profiles for POEGEA-*b*-P(DAPA-*co*-TFEA)-*b*-POEGEA@HCFU/IR780 micelles in PBS buffer at pH 7.5, 6.5 and 5.5.

Biocompatibility of micelles

Hela, B16-F10 and 3T3 cells were selected as models to test the biocompatibility of neat POEGEA-*b*-P(DAPA-*co*-TFEA)-*b*-POEGEA and POEGEA-*b*-PTFEA-*b*-POEGEA (**Fig. S6**). Hela cells were seeded into a 96-well plate at a density of 1×10^4 cells/well and grown in CO_2-air (5%/95%) for overnight. Then, the Hela cells were incubated with different concentrations of two polymer materials (100, 150, 200, 300 μg/mL) for 24 h. Subsequently, the CCK8 solution were added and incubated with cells for another 1 h. Cells viability was determined through microplate reader at 450 nm. The same experimental methods were utilized to test

the biocompatibility of B16-F10 and 3T3 cells.

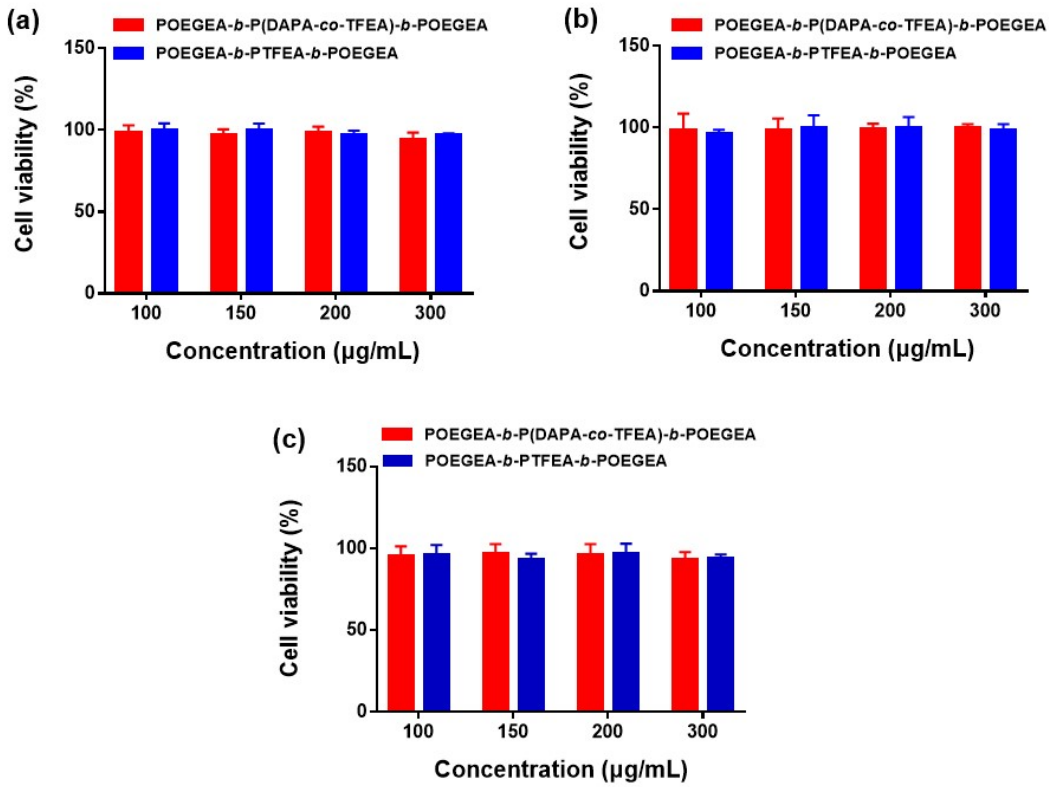

Fig. S6: Biocompatibility of neat POEGEA-*b*-P(DAPA-*co*-TFEA)-*b*-POEGEA and POEGEA-*b*-PTFEA-*b*-POEGEA toward Hela (a), B16-F10 (b) and 3T3 (c) cells.

In vitro cytotoxicity assay

To explore the advantages of H-bonded supramolecular micelles on tumor growth inhibition, the dispersions with HCFU concentrations of 0.5, 1, 2, 4, 6 μg/mL in POEGEA-*b*-P(DAPA-*co*-TFEA)-*b*-POEGEA@HCFU/IR780 or POEGEA-*b*-PTFEA-*b*-POEGEA@HCFU/IR780 were incubated with Hela cells for 24 h in 96-well plates in the dark. Thereafter, the dispersions were substituted by CCK8 solution and incubated for 1.5 h. Then, cells viability was determined by microplate reader at 450 nm. At the same time, the free HCFU and IR780 with the same concentrations were also considered.

To study the combined effect of HCFU and IR780 mediated by POEGEA-*b*-P(DAPA-*co*-

TFEA)-*b*-POEGEA@HCFU/IR780 or POEGEA-*b*-PTFEA-*b*-POEGEA@HCFU/IR780, the Hela cells were incubated with the dispersions with HCFU concentrations of 0.5, 1, 2, 4, 6 μg/mL for 6 h in the dark. After the 96-well plate was irradiated with 808 nm laser (0.6 W/cm^2, 3 min), it was continued to incubate to 24 h. Finally, cytotoxicity was quantified via CCK8 assays after another 1.5 h of incubation.

Cellular uptake of micelles

In vitro cellular uptake of POEGEA-*b*-P(DAPA-*co*-TFEA)-*b*-POEGEA@HCFU/IR780 micelles were then studied by using Hela cells. Fluorescein isothiocyanate (FITC) (2%, w/w) was doped into nanoparticles to obtain POEGEA-*b*-P(DAPA-*co*-TFEA)-*b*-POEGEA@HCFU/IR780/FITC micelles by using phacoemulsification method. HeLa cells were cultured in high glucose medium Dulbecco's Modified Eagle Medium (DMEM, Gibco) with 10% fetal bovine serum (FBS) and penicillin-streptomycin solution (10 mg·mL^{-1} of streptomycin and 10000 U·mL^{-1} of penicillin) at 37 ^{o}C in an atmosphere of 5% carbon dioxide and 95% humidified air. For confocal laser scanning microscope (CLSM) , HeLa cells (2 x 10^3 cells/well) were seeded in confocal dishes for 24 h, and then the DMEM medium was replaced with fresh DMEM containing POEGEA-*b*-P(DAPA-*co*-TFEA)-*b*-POEGEA@HCFU/IR780/FITC micelles (10 μg·mL^{-1}) to incubate for 1h, 2h, 4h, 8h respectively. Hoechst-33324 was used as a control for nuclear staining. Confocal images were observed under a high-resolution confocal fluorescence microscope with excitation wavelength of 405 nm for Hoechst-33324, and 488 nm for POEGEA-*b*-P(DAPA-*co*-TFEA)-*b*-POEGEA@HCFU/IR780/FITC micelles. For flow cytometry measurement, HeLa cells were

seeded into 12-well cell culture plates and incubated at 37 °C for 24 h and 5% carbon dioxide. (equivalent cell density of 10000 per well). After that, POEGEA-*b*-P(DAPA-*co*-TFEA)-*b*-POEGEA@HCFU/IR780/FITC micelles was added into wells at the final concentration of 10μg·mL^{-1} and incubated for 1, 2, 4, 8h, respectively. Subsequently, the cells were trypsinized and washed with PBS for flow cytometry analysis. Flow-Jo software was used to measure the fluorescence intensity of the cells suspended in PBS.

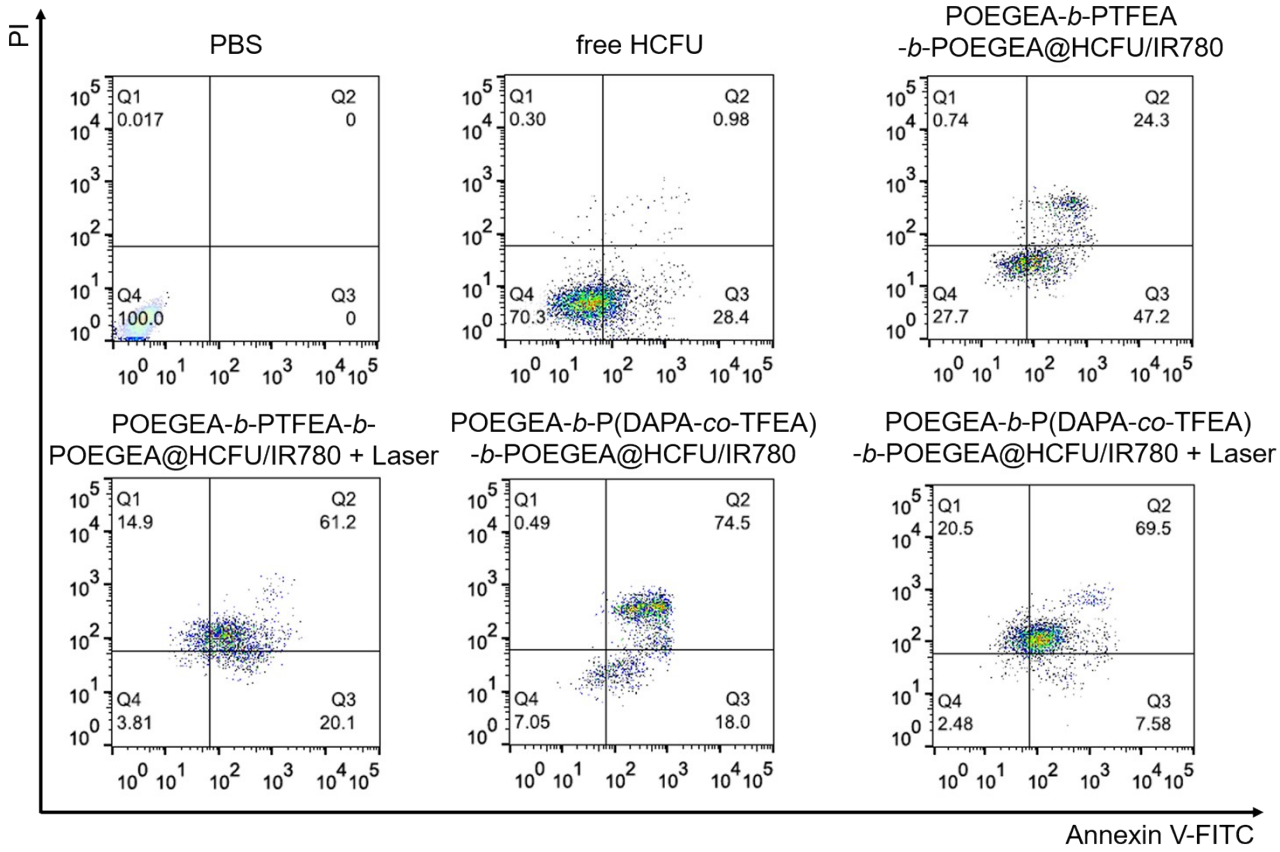

Fig. S7: Cellular uptake analyses of free HCFU, POEGEA-*b*-PTFEA-*b*-POEGEA@HCFU/IR780, POEGEA-*b*-P(DAPA-*co*-TFEA)-*b*-POEGEA@HCFU/IR780 micelles with/without laser irradiation by flow cytometry toward HeLa cells after co-incubation.

Live/dead assay of HeLa cells

The live/dead assay was employed to evaluate the cytotoxicity of POEGEA-*b*-P(DAPA-*co*-

TFEA)-*b*-POEGEA@HCFU/IR780 micelles with or without laser irradiation. Typically, HeLa cells were seeded in 96-well plates for 24 h, and POEGEA-*b*-P(DAPA-*co*-TFEA)-*b*-POEGEA@HCFU/IR780 micelles (10 μg·mL^{-1}) were added into the plates for another 8 h incubation. After that, the cells were handled with laser irradiation for 5 min and then incubated for another 12 h in the chamber slides. According to the instruction manual, the cells were stained with Calcein-AM and PI for 30 min. After staining, the fluorescent images were obtained via a fluorescence microscope.

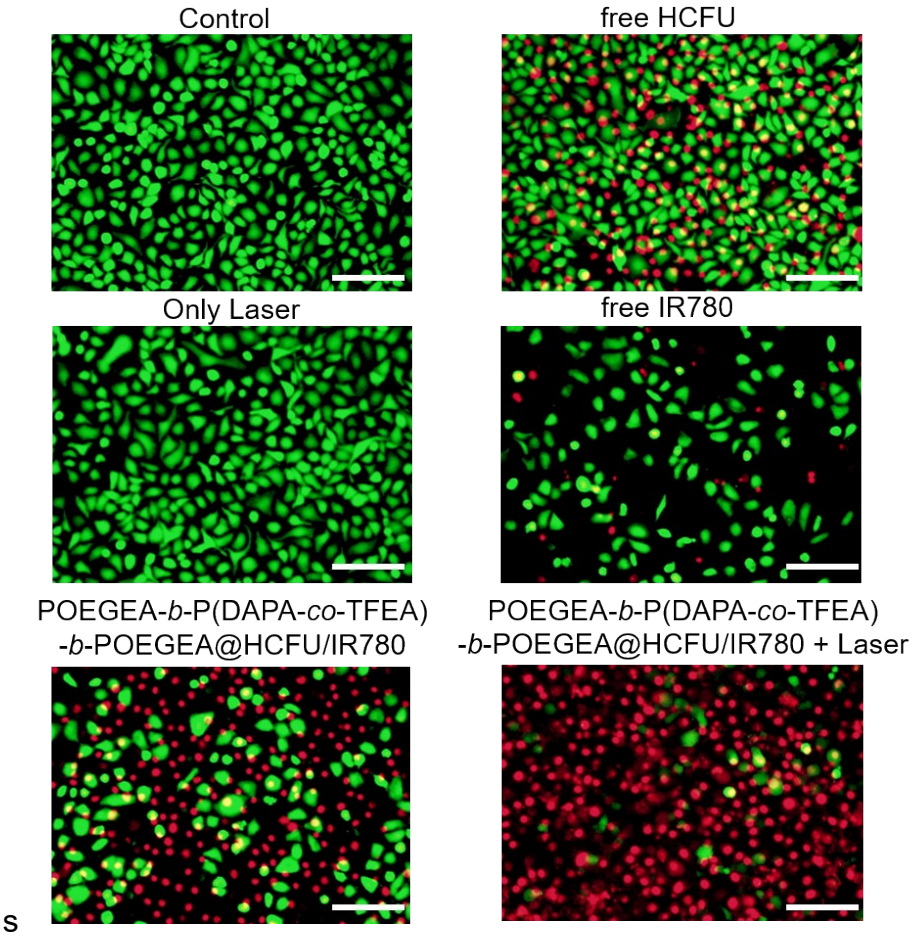

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Fig. S8: Live/dead analyses of HeLa cells after incubation with free HCFU, IR780 and POEGEA-*b*-P(DAPA-*co*-TFEA)-*b*-POEGEA@HCFU/IR780 micelles, and followed by laser irradiation. Scale bars represent 100 μm.

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