Supporting Information

Dynamic Intracellular Tracking Nanoparticles via pH-evoked "Off-On" Fluorescence

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Experimental Section

Materials. Poly(ethylene glycol) (Mw=2000 g · mol^{-1}) (PEG2k) was purchased from Sigma-Aldrich Co. (Steinheim, Germany) and used as received. Methacryloyl chloride, 1-(2-Hydroxyethyl)-3, 3-dimethylindolino-6'-nitrobenzopyrylospiran (SP-OH), 2-Bromoisobutyryl bromide, and N, N, N', N'', N''-Pentamethyldiethylenetriamine (PMDETA) were purchased from TCI (Japan). CuBr, petroleumether, ether, methylbenzene and triethylamine (Et_3N) were purchased from KelongChemical Co. (Chengdu, China) and used without further purification. Dichloromethane (CH_2Cl_2) and tetrahydrofuran (THF) were distilled before use. Dulbecco's Modified Eagle's Medium (DMEM), Roswell Park Memorial Institute (RPMI) 1640 medium, fetal bovine serum (FBS) and penicillin-streptomycin were used for cell tests. Human Cervical carcinoma cell (Hela) and human hepatoma cell (HepG2) were purchased from the Chinese Academy of Science Cell Bank for Type Culture Collection (Shanghai, China). LysoTracker Green DND-2 was purchased from invitrogen. All the other reagents and solvents were analytical grade and used as received.

Synthesis of SPMA. SP-OH (0.5 g, 1.42 mmol) and Et_3N (295 μL, 2.13 mmol) were both dissolved in dry CH_2Cl_2 solution (15 mL) under N_2 atmosphere, and then the mixture was cooled to 0 °C in the dark environment. Methacryloyl chloride (206 μL, 2.13 mmol) in dry CH_2Cl_2 (5 mL) was added dropwise over 1.0 h. After stirring for 24 h at 0 °C, the solution was filtered, and the solvent was removed using a rotary evaporator. The residues were purified by silica gel column chromatography (200-300 mesh) using petroleumether/CH_2Cl_2 (4:1v/v) as eluent.

Synthesis of Br-PEG-Br. 2.0 g of poly(ethylene glycol) (HO-PEG-OH) and 0.69 g of 2-Bromoisobutyryl bromide (371 μL, 3.0 mmol) were dissolved in 50 mL dry methylbenzene under N_2 atmosphere. The mixture was then cooled to 0 °C. When the mixture became clear, Et_3N (415 μL, 3.0 mmol) in 10 mL dry methylbenzene was slowly added dropwise into the solution at 0 °C for 1.0 h. The mixture was then stirred at room temperature for 48 h. After that the crude products were precipitated in cold ether for three times. The precipitate was dried in a vacuum oven at room temperature for 72 h.

Synthesis of SPMA-PEG-SPMA. SPMA-PEG-SPMA was synthesized by atom transfer radical polymerization (ATRP) of SPMA using bromoterminated poly (ethylene glycol) (Br-PEG-Br) as macro-initiator. 0.5 g of Br-PEG-Br and SPMA (1.05 g, 2.5 mmol) were dissolved in 20 mL dry THF. Oxygen was removed by three freeze–pump–thaw cycles, and a mixture of CuBr (0.36 g, 2.5 mmol) and PMDETA (0.43 g, 2.5 mmol) dissolved in 0.5 mL THF was added to the solution under N_2 atmosphere. The mixture solution was conducted at 45 °C for 24 h. The reaction was stopped by opening the flask to air, and the catalyst was removed by passing the solution through a neutral alumina column. The copolymer solution was precipitated in cold ether for three times and dried under vacuum oven.

Characterization of copolymers. ^1H NMR spectra were recorded on a Bruker 400 MHz spectrometer. Samples were dissolved in $CDCl_3$ with tetramethylsilane as the internal standard. The molecular weights and weight distributions of the synthetic polymers were determined by gel permeation chromatography (GPC). GPC measurement was carried out on an Agilent 1100 Series with THF as eluent. Molecular weights were calibrated on PS standards.

Preparation of SPMA-PEG-SPMA nanoparticles. SPMA-PEG-SPMA nanoparticles were prepared by solvent evaporation method. Briefly, 1 mL of SPMA-PEG-SPMA polymer (20 mg) solution in THF was added dropwise (1 drop every 10s) to 10 mL deionized water. After stirring for 24 h until the organic solvent volatilized, the resulting solution was filtered

through a 450 nm syringe filter (Milipore, Garrigtwohill, Co. Cork, Ireland).

Characterization of SPMA-PEG-SPMA nanoparticles. The mean diameter and size distribution of the nanoparticles were determined by dynamic light scattering (DLS, Malvern Zetasizer Nano ZS). Each sample was filtered through a 450 nm syringe filter before analysis. The scanning electron microscope (SEM, S-4800, HITACHI) was used to observe the morphology of micelles.

pH triggered isomerization of SP to MC. To investigate acid-triggered spectral response of SPMA-PEG-SPMA nanoparticles, the micellar stock solution was diluted to 0.6 mg/mL by adding acetate buffer solutions with different pHs 7.4, 6.0 and 5.0 respectively. At definite time intervals, the micellar solutions were immediately measured by fluorescence spectrophotometer with a FLS 920 spectro photometer (Edinburg) in dark environment. The wavelength scan rate was designed at 1200 r/min. The excitation wavelength is 420 nm, and the emission spectrum was collected the wavelength from 450 to 700 nm.

Intracellular tracking of nanoparticles. Confocal laser scanning microscopy (CLSM) was employed to track the internalization and localization of SPMA-PEG-SPMA nanoparticles in cells. HepG2 and Hela cells were seeded on glass dishes at a cell density of 1×10^4 mL^{-1}. After incubating for 24 h, the culture medium was removed. 200 μL of micellar solution was added into each dish, followed by further incubation for 2, 4 and 6 h at 37 °C. Subsequently, Lyso-Tracker Green (100 nM) was added after removal of culture medium and the cells were incubated for another 30 min. The dishes were rinsed with ice cold PBS (pH = 7.4) to protect the attached LysoTracker Green, and then observed by confocal laser scanning microscopy (CLSM, Leica TCP SP5). The excitation wavelengths were 504 nm and 480 nm for Lyso-Tracker Green and nanoparticles, respectively, accompanying with corresponding emission wavelengths of 511 nm and 620 nm[1].

Cytotoxicity assay. Mouse fibroblasts NIH 3T3, liver cancer cells HepG2 and cervical cancer cells Hela were cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. The cytotoxicity of blank nanoparticles was tested by Cell Counting Kit-8 assay (CCK-8, Dojindo, Japan) against 3T3, HepG2 and Hela cells. Cells at a logarithm phase were seeded in 96-well plates with the cell density of 4×10^3 mL^{-1}. Each well was cultured with 100 μL medium. After 24 h incubation, the culture medium was removed and replaced with 100 μL fresh medium containing blank nanoparticles with different concentration. Then they were incubated for another 48 h. The culture medium was removed and the wells were rinsed with PBS (pH = 7.4) for three times. 100 μL CCK-8 (volume fraction 10%) solution was added to each well. After incubated for 2 h, the absorbance was measured at a Thermo Scientific MK3 (Thermo fisher, US) at the wavelength of 450 nm. The cell viability was calculated according to the following formula:
Cell viability (%) = (OD value of test group-OD value of blank group) / (OD value of control group-OD value of blank group).

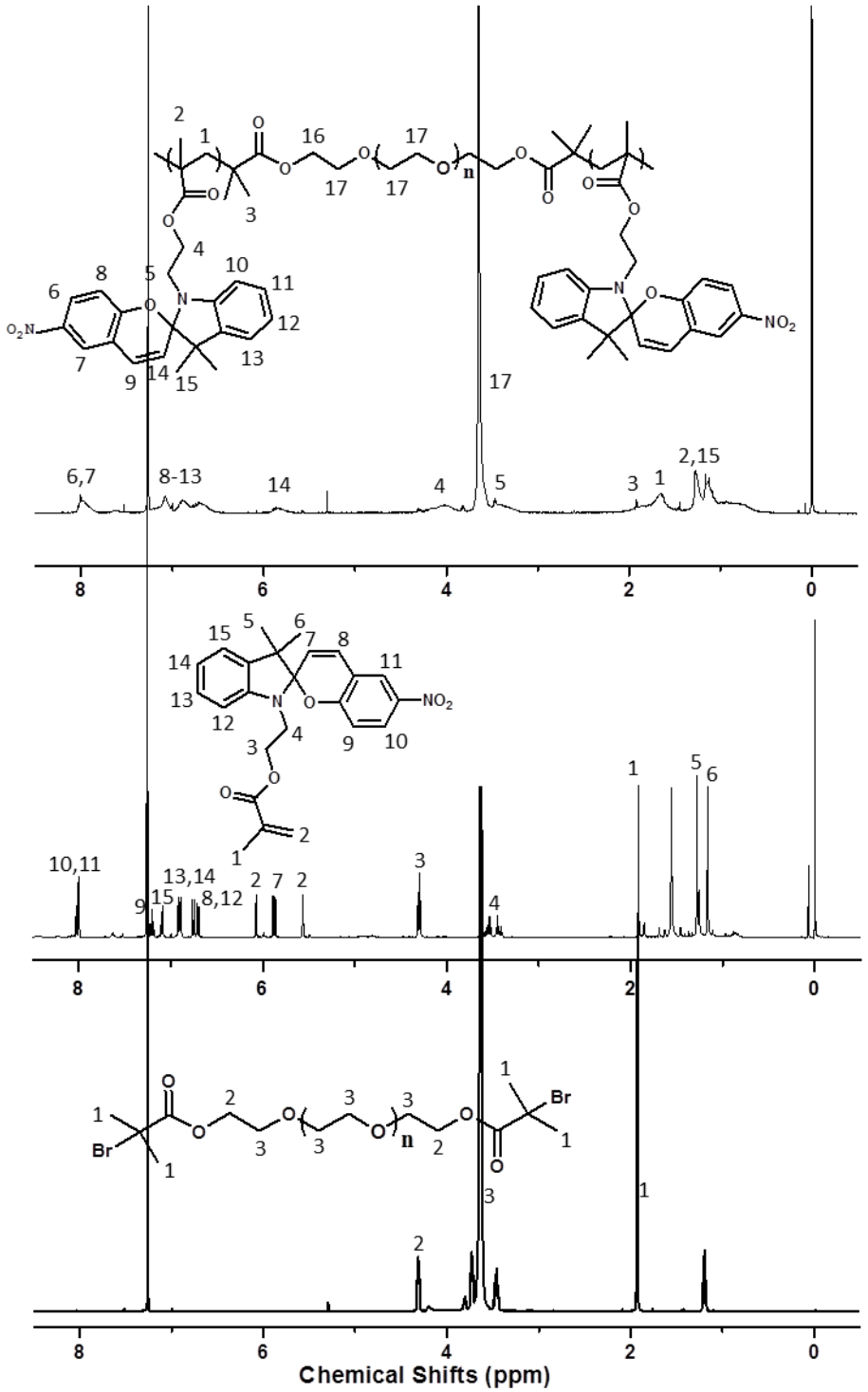

Figure S1. [1]HNMR spectra of (a) Br-PEG-Br (b) SPMA and (C) SPMA-PEG-SPMA copolymers.

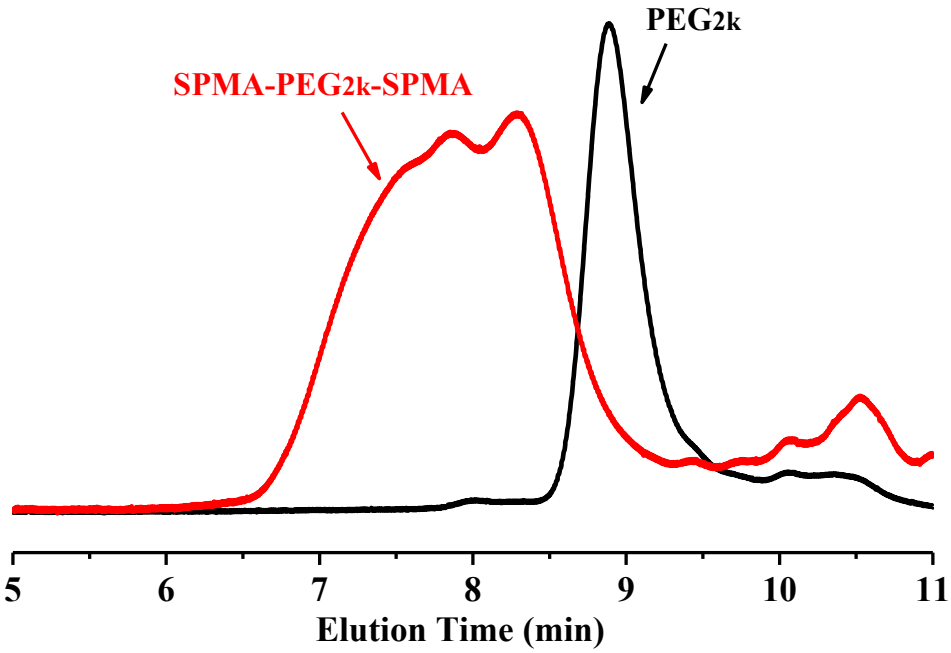

Figure S2. The GPC traces of PEG2k and SPMA-PEG-SPMA copolymers.

Table S1. The properties of SPMA-PEG-SPMA copolymers

Sample	Molecular weight			Micelle diameter (nm)
	Mn	Mw	Mw/Mn	
PEG2k	2469	2805	1.136	--
SPMA-PEG2k-SPMA	7695	13973	1.816	98

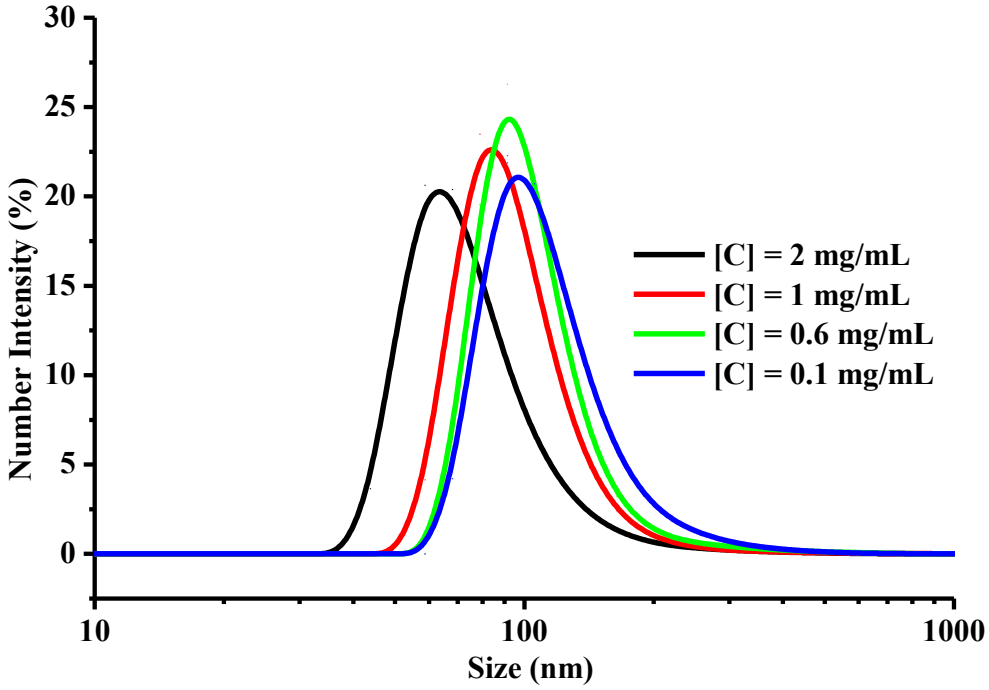

Figure S3. The size of copolymer nanoparticles at different micellar concentrations determined by DLS.

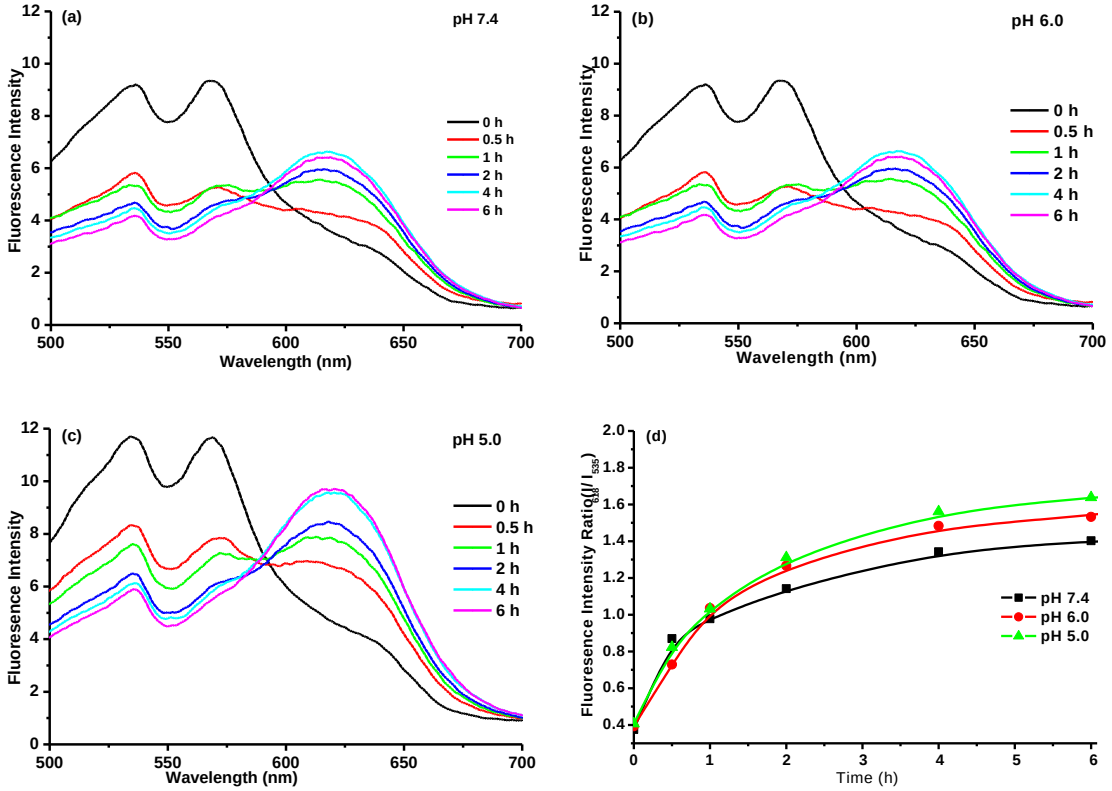

Figure S4. The fluorenence intensities of polymeric NPs in different pH media versus incubation time.

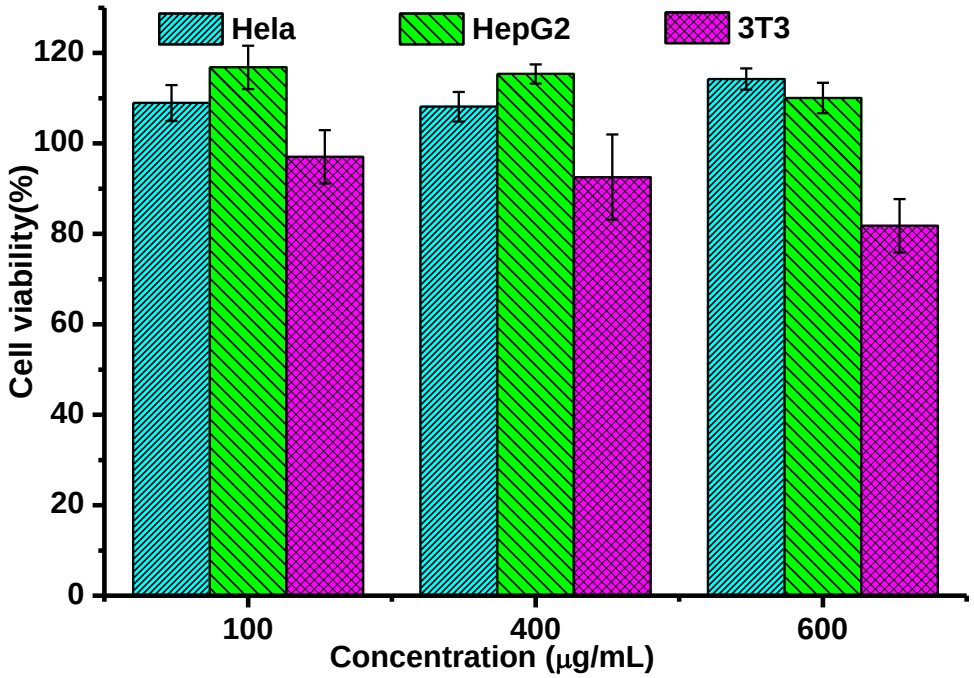

Figure S5. Cell viability of SPMA-PEG-SPMA nanoparticles at different micellar concentration after incubated with different cancer cells.

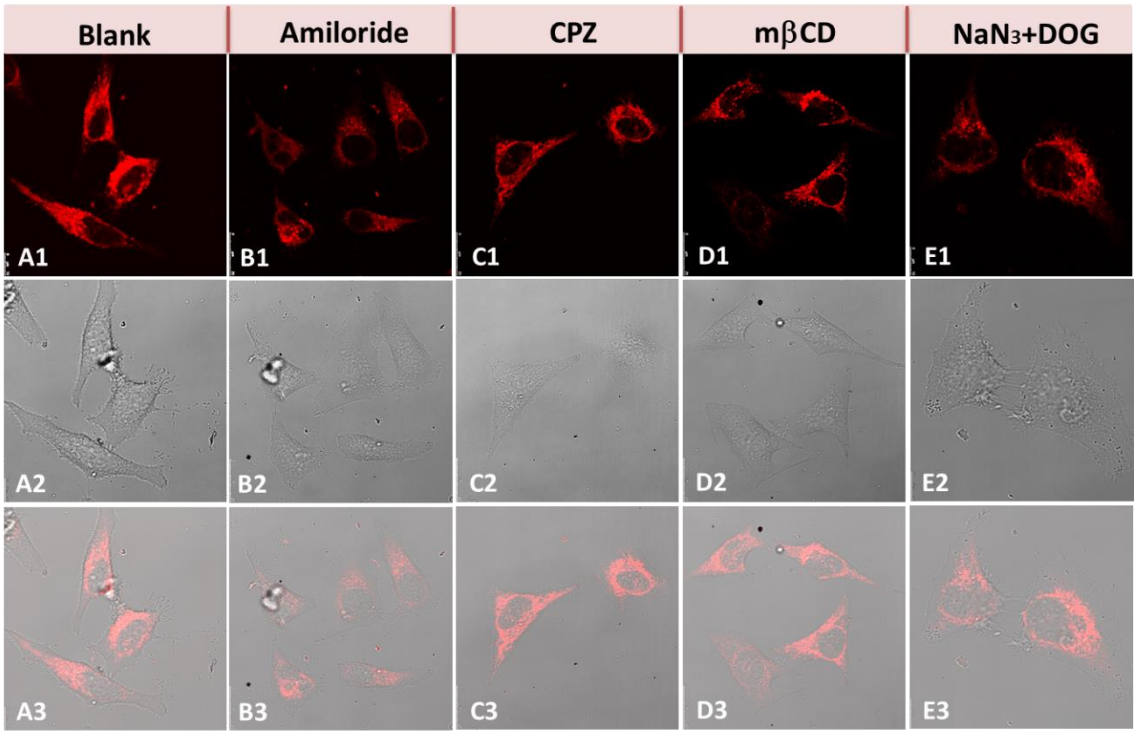

Figure S6. The endocytic pathways of SPMA-PEG-SPMA nanoparticles in Hela cells. The confocal microscopy images of cells with different inhibitors. A: Control; B: amiloride (50 μM); C: chlorpromazine; D: mβCD (7 mM); E: NaN_3/DOG. The images from up to down were cells in fluorescence (1), bright field (2) overlay images (3).

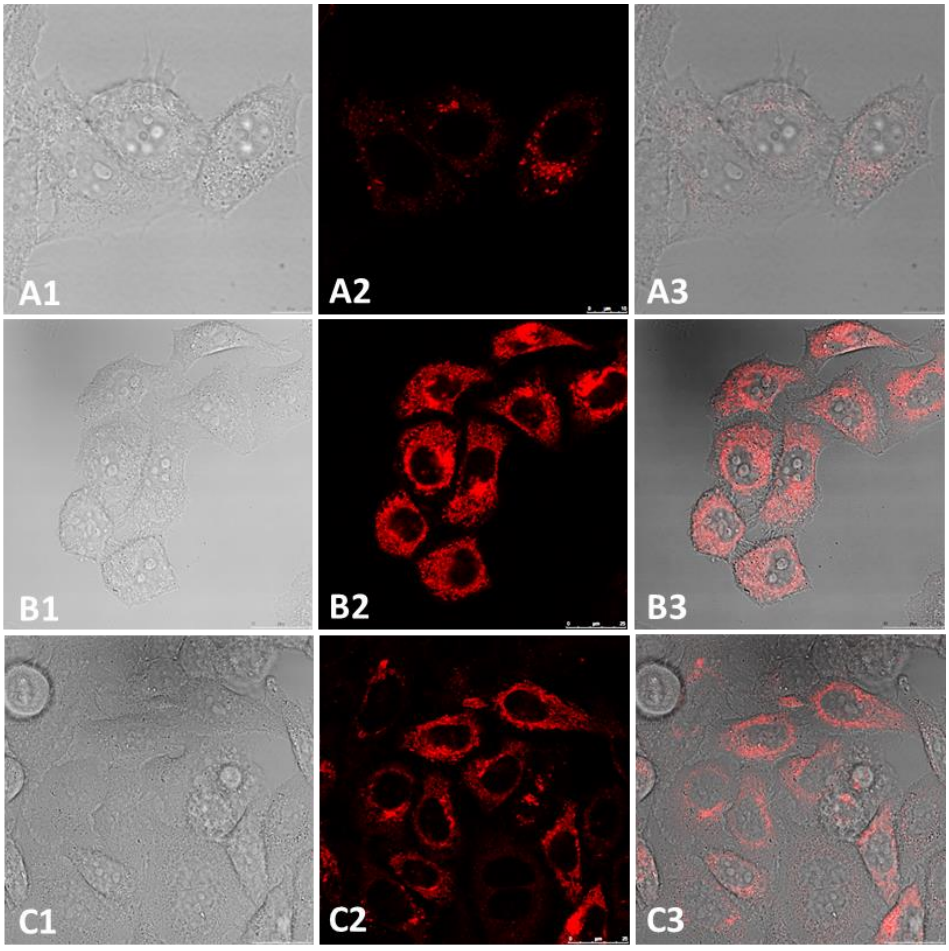

Figure S7. Fluorescence of SPMA-PEG-SPMA nanoparticles with different micellar concentration (A) 0.1 (B) 0.6 (C) 1 mg/mL emerging in HepG2 cells for 6 h incubation, the images from left to right (1 to 3) were bright field, red fluorescence and overlay.

1. J. Cao, X. Xie, A. Lu, B. He, Y. Chen, Z. Gu and X. Luo, *Biomaterials*, 2014, **35**, 4517-4524.