

**Materials:** Boc-NH-PEG-SCM (2k Da) was purchased from Creative PEGWorks and used as received. *N,N,N',N'',N'''*-pentamethyldiethylenetriamine (PMDETA) and propargylamine were purchased from Alfa aesar and used as received. Doxorubicin hydrochloride (Dox · HCl) was purchased from Zhejiang Hisun Pharmaceutical Co., Ltd. (China). Camptothecin (CPT) and Pyrene were purchased from Aladdin-reagent (China) and used as received. CuBr was purchased from Sigma-Aldrich and used as received. *N,N*-dimethyl formamide (DMF) and dimethyl sulfoxide (DMSO) were dried over 4 Å molecular sieve and distilled over anhydrous MgSO<sub>4</sub>. Dichloromethane (DCM) was distilled over CaH<sub>2</sub> before used. Bovine serum albumin (BSA), Dubelcco's Modified Eagle's Medium (DMEM), Roswell Park Memorial Institute medium 1640 (RPMI-1640), penicillin–streptomycin, trypsin, phosphate-buffered saline (PBS), Hoechst 33258, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from GIBCO Invitrogen Corporation. Dox-N<sub>3</sub> and biotin-NHS were synthesized according to a previous work.<sup>1</sup>

#### Boc-NH-PEG-Alkyne

Boc-NH-PEG-SCM (1.00 g, 0.5 mmol) in 30 mL of DCM was stirred at 0°C and propargylamine (0.275 g, 5 mmol) was added dropwise into the solution. Then the reaction mixture was warmed to room temperature and stirred for 24 h. After reaction, 20 mL of DCM was added and the solution was washed with distilled water twice, brine once, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and filtrated. The filtrate was concentrated

by rotary evaporation and added into excess ice-cooled ether to obtain white solid. The crude product was further precipitated twice in ether. The Boc-NH-PEG-Alkyne was obtained as a white powder. Yield: 862 mg (88.7%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>, δ ppm): 7.59 (t, 1H), 5.04 (t, 1H), 4.09 (m, 2H), 4.02 (s, 2H), 3.87 (m, 2H), 3.64 (m, 148H), 3.51 (m, 2H), 3.43 (m, 2H), 3.31 (m, 2H), 2.24 (s, 1H), 1.44 (s, 9H).

#### Biotin-PEG-Alkyne (BPA)

Boc-NH-PEG-Alkyne (0.80 g, 0.412 mmol) was dissolved in 20 mL DCM with 50% of TFA. The solution was stirred at room temperature for 2 h and the solvent was evaporated to a small volume under vacuum. The residue was precipitated in excess ice-cooled ether for three times. The obtained yellowish NH<sub>2</sub>-PEG-Alkyne was used in next step without further purification. Biotin-NHS (155 mg, 0.454 mmol) and NH<sub>2</sub>-PEG-Alkyne were dissolved in 20 mL of DCM, following with triethylamine (TEA, 45.9 mg, 0.454 mmol). The mixture was stirred for 48h at room temperature and then washed with distilled water, brine and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. After filtration, the filtrate was concentrated and precipitated in ice-cooled ether. The crude product was purified by dialysis against deionized water (Mw 1000, cutoff). Yield: 628 mg (73.8%).

#### Biotin-PEG-Dox (BPD)

Biotin-PEG-Alkyne (200 mg, 0.097 mmol) and Dox-N<sub>3</sub> (80 mg, 0.118 mmol) were dissolved in 2 mL of DMF. After three freeze-pump-thaw cycles, PMDETA (27.7 mg,

0.160 mmol) and Cu(I)Br (7.00 mg, 0.049 mmol) were added and the solution was stirred under Ar atmosphere at 35°C for 24 h. The reaction was stopped by opened to air, and the mixture was diluted with THF and passing through a short alumina column to eliminate the copper. The crude product was extensively dialyzed against DMSO for 72 h and then against deionized water for 24 h (cutoff Mw 1000). Yield: 187 mg (71.2%).

## Methods

<sup>1</sup>H NMR spectra were recorded at 300 MHz on a Mercury VX-300 spectrometer by using tetramethylsilane (TMS) as the internal reference. Infrared spectra were recorded on a Nicolet Avator 360 FT-IR spectrometer. Size and distribution measurements were performed on a Nano-ZSZEN3600 (Malvern) instrument at room temperature. The molecular weight and molecular weight distribution of the polymer were determined by gel permeation chromatography equipped with a Waters 2690 separation module and a Waters 2410 refractive index detector. DMF was used as eluent at a flow rate of 0.5 mL min<sup>-1</sup> with the temperature maintained at 30°C and the results were calibrated against with polyethylene glycol standards. The TEM images were taken by JEM-2100 (HR) transmission electron microscope at an acceleration voltage of 200 keV.

The surface tensions of a series of BPD aqueous solutions with concentrations ranging from 4×10<sup>-1</sup> to 4×10<sup>-4</sup> mg/mL were measured by pendant drop method on an optical contact angle measuring device (OCA 30, Dataphysics). The critical aggregation

concentration (CAC) of BPD was determined based on the surface tensions at low and high concentration regions.

The content of Dox in BPD was determined using fluorescence spectroscopy. The BPD was resolved in 1N HCl aqueous solution and the solution was kept for 24 h at room temperature in dark. The content of Dox was calculated based on the fluorescence intensity at emission wavelength of 560 nm, excitation wavelength of 488 nm, and slit width of 5 nm calibrated by a standard curve of Dox·HCl.<sup>2</sup>

Prodrug/drug complexes (BPD/Dox and BPD/CPT) were prepared by dialysis. Typically, 1.0 mg of BPD and 0.1 mg model drugs (Dox was pretreated with TEA) was stirring in 200  $\mu$ L of DMSO, and then 1.8 mL of DI water was added dropwise into the above solution. After 4 h of stirring at room temperature, the mixture was dialyzed (Mw 1000, cutoff) against with DI water to get rid of organic solvent and unencapsulated drugs. Then the mixture was passing through a 0.45  $\mu$ m filter and lyophilized. The amount of the loaded model drugs were calculated based on the UV absorbance at 488nm (Dox) and 368 nm (CPT) calibrated with standard stocks in DMSO.

Cell internalization was observed on a confocal laser scanning microscopy (CLSM, Nikon C1-si). HeLa cells were seeded in a 35 mm cell culture dish with glass bottom and incubated at 37°C for 24 h. The samples in DMEM with 10 % fetal bovine serum (FBS) were added to replace the medium. After 1 h of incubation, the medium was removed and the cells were washed 3 times with PBS. Then the nuclear was stained with Hoechst 33258 and the cells were fixed with 4 wt % formaldehyde in PBS for 20

min at room temperature. A drop of mounting media (10% PBS, 90% glycerol) were added to mount the cells. The fluorescence was examined under excitation at 405 nm for Hoechst 33258 and 488 nm for Fluorescein and Dox. The CLSM was also used to observe the influence of the BPD/CPT complex on the cell culture. The experimental process was similar to the cell internalization measurement but the incubation time of samples with cells was set to 4h, 12h and 24h, respectively.

The cytotoxicity assessment was carried out in Hela cells and Ht-29 cells by using the MTT assay. 100  $\mu$ L of cell suspension containing  $5 \times 10^3$  cells were seeded into each well of a 96-well plate and incubated at 37°C with 5% CO<sub>2</sub> for 24 h. Then the cells were treated with samples at various concentrations and carried out a further incubation for 48 h. After that, the mediums was replaced with 200  $\mu$ L of fresh mediums. 20  $\mu$ L of MTT solution in PBS (5 mg/mL) was added into each well and the cells were incubated for another 4 h. The medium in each well was carefully removed and replaced by 100  $\mu$ L DMSO. When the purple solution was homogeneous, the absorbance at 570 nm was recorded by a microplate reader (Multiskan GO, Thermo Fisher). Cell viability was calculated by

$$\text{Cell viability (\%)} = (A_{\text{treated}}/A_{\text{control}}) \times 100\%$$

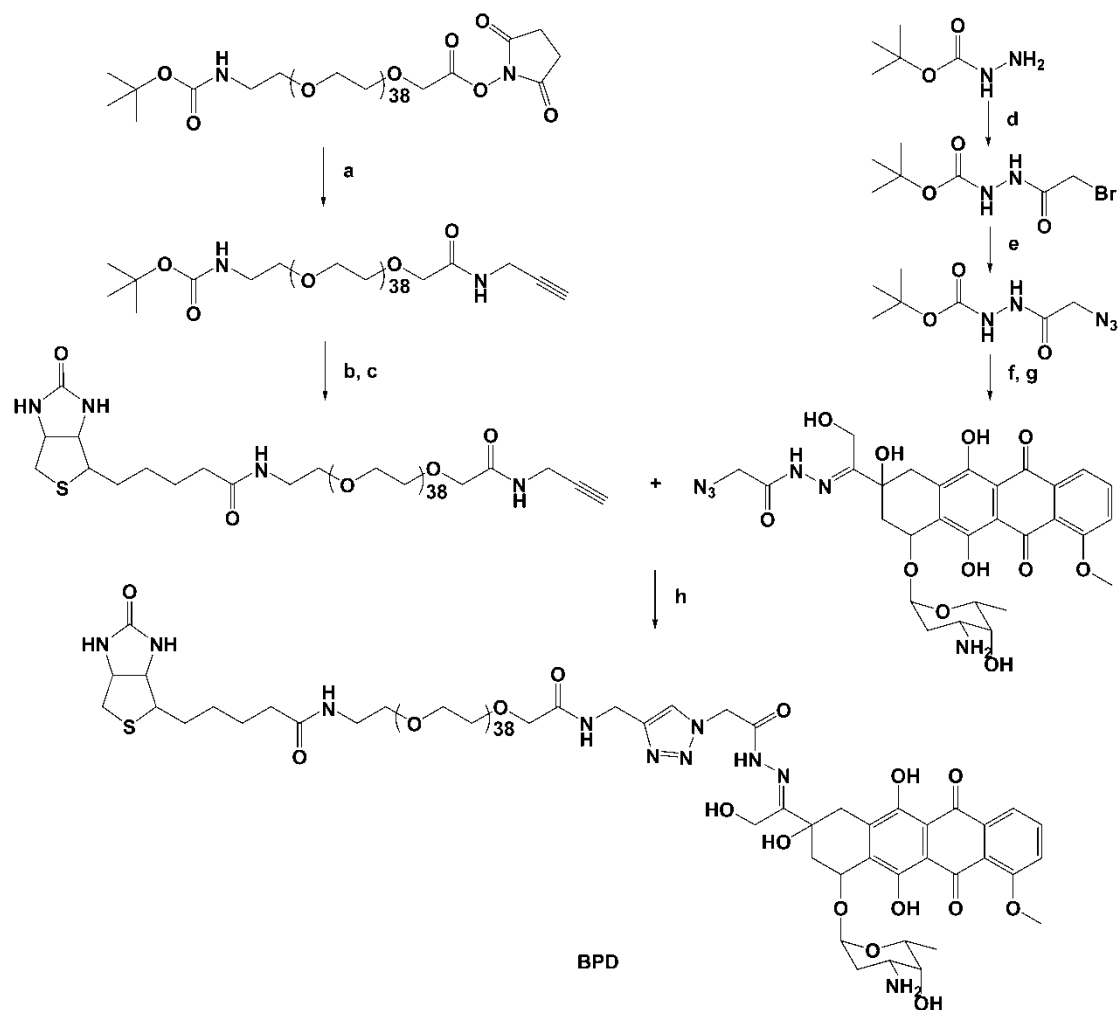
The data are shown as the average value  $\pm$  standard deviation.

#### Reference

- 1 Z. Yuan, Z. Que, S. Cheng, R. Zhuo and F. Li, *Chem. Commun.*, 2012, **48**, 8129.
- 2 L. Zhou, R. Cheng, H. Tao, S. Ma, W. Guo, F. Meng, H. Liu, Z. Liu, and Z. Zhong, *Biomacromolecules*, 2011, **12**, 1460

Table S1 The IC<sub>50</sub> values (µg/mL)

Cell lines	BPD	BPD/Dox	BPD/CPT	Dox	CPT
HeLa	20.69	9.72	0.74	0.72	0.33
Ht-29	14.69	9.66	0.59	0.32	0.07
Cos7	30.73	17.15	2.05	0.18	0.02



Scheme S1. The synthesis route of BPD. (a) propargylamine and TEA, in CH<sub>2</sub>Cl<sub>2</sub>, rt. 24 h; (b) 50% TFA in CH<sub>2</sub>Cl<sub>2</sub>, rt. 2 h; (c) Biotin-NHS and TEA, in DMF, rt. 48 h; (d) bromoacetyl bromide, TEA, ice cool to rt. 24 h; (e) NaN<sub>3</sub>, DMF, 40°C, 24 h; (f) 50% TFA in CH<sub>2</sub>Cl<sub>2</sub>, rt. 4 h; (g) Dox•HCl, catalytic amount TFA, reflux in anhydrous CH<sub>3</sub>OH, 48 h in dark; (f) CuBr, PMDETA, in DMF, three freeze-pump-thaw cycles, 35°C, 24 h, dialysis against DMSO for 72 h and then DI water for 24 h.

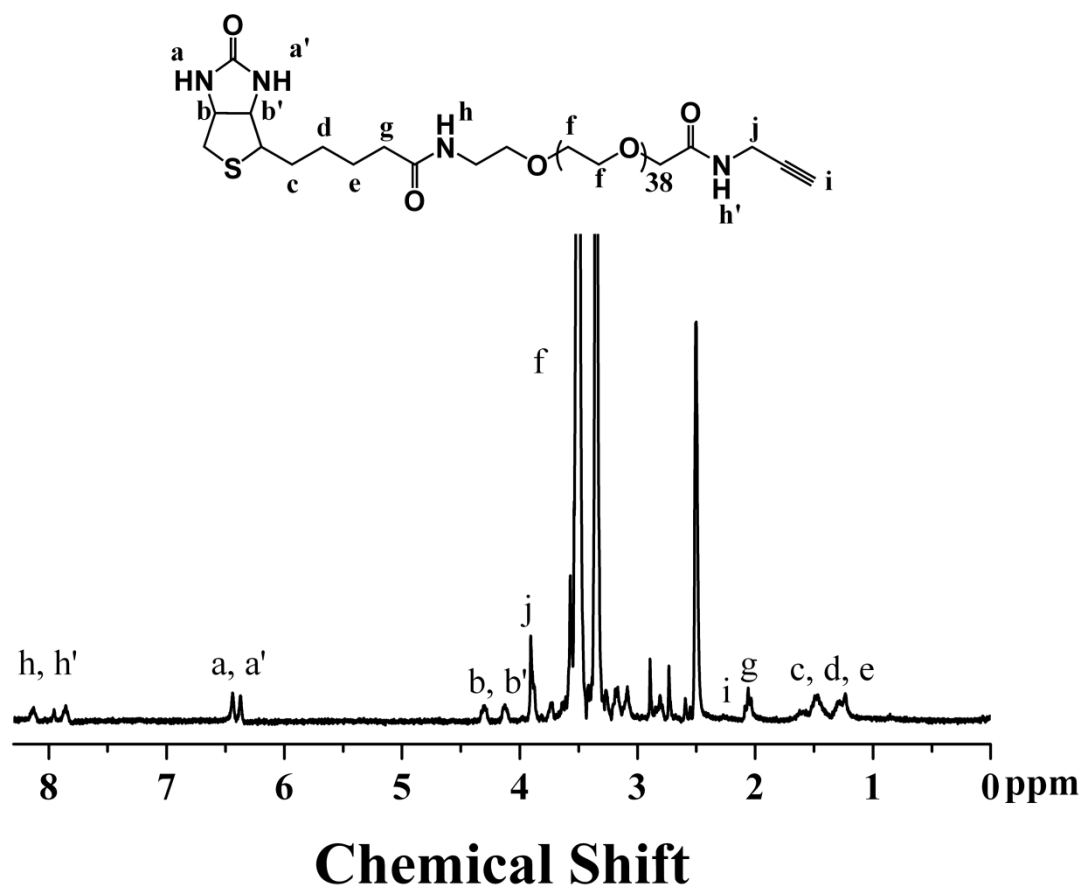


Figure S1. The <sup>1</sup>H NMR spectrum of the Biotin-PEG-Alkyne.



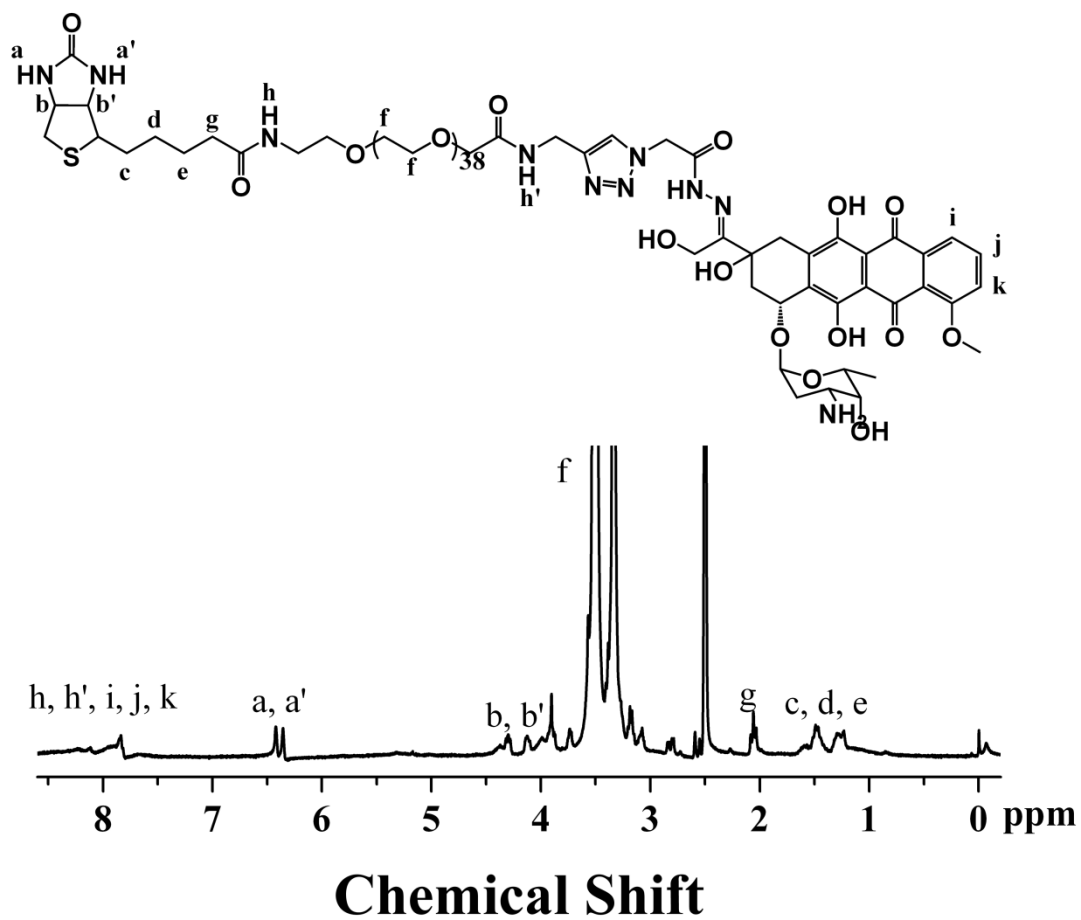


Figure S2. <sup>1</sup>H NMR spectrum of Biotin-PEG-Dox.

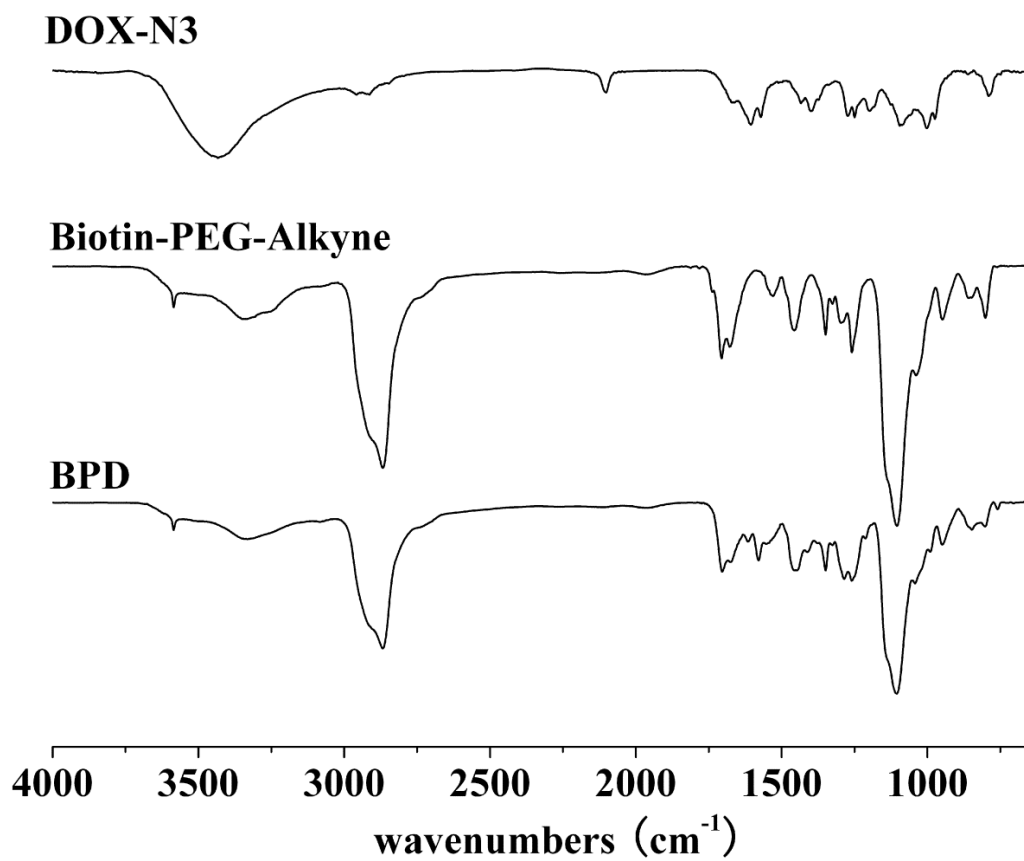


Figure S3. Change of FT-IR spectra in the synthesis of BPD.

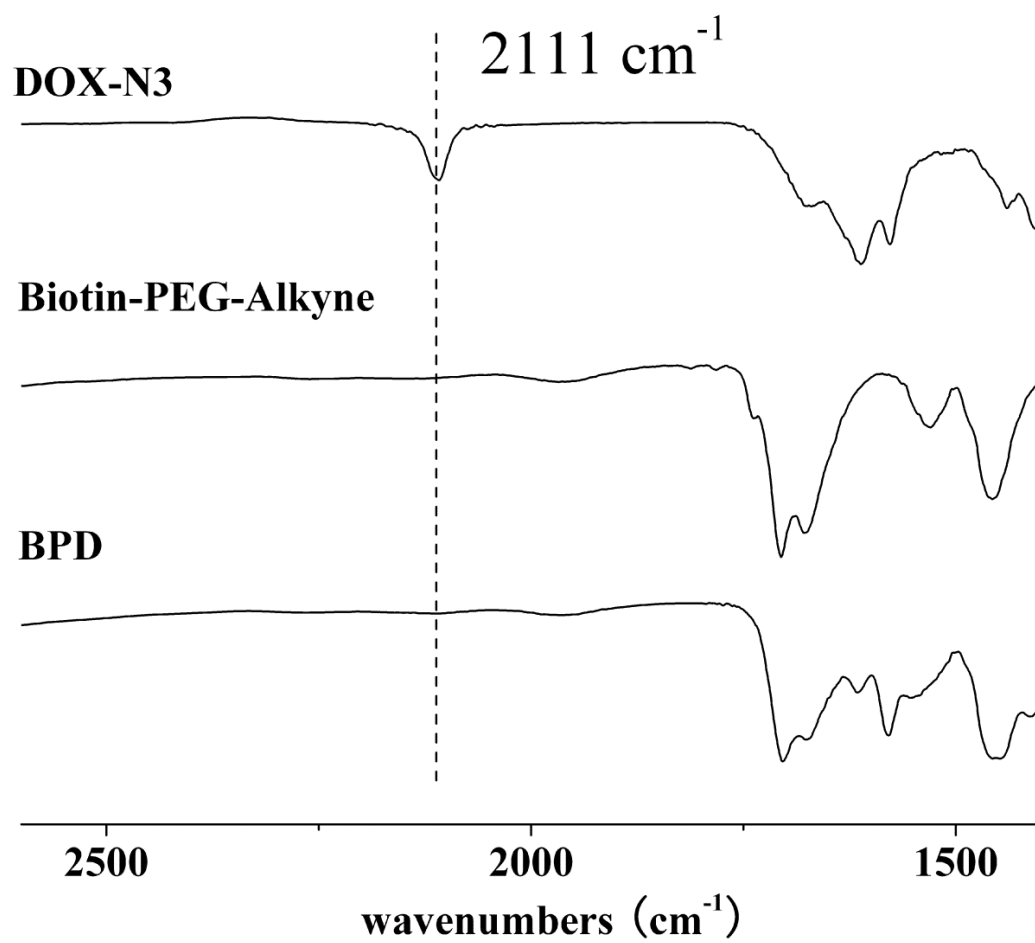


Figure S4. Enlargement of Figure S3.

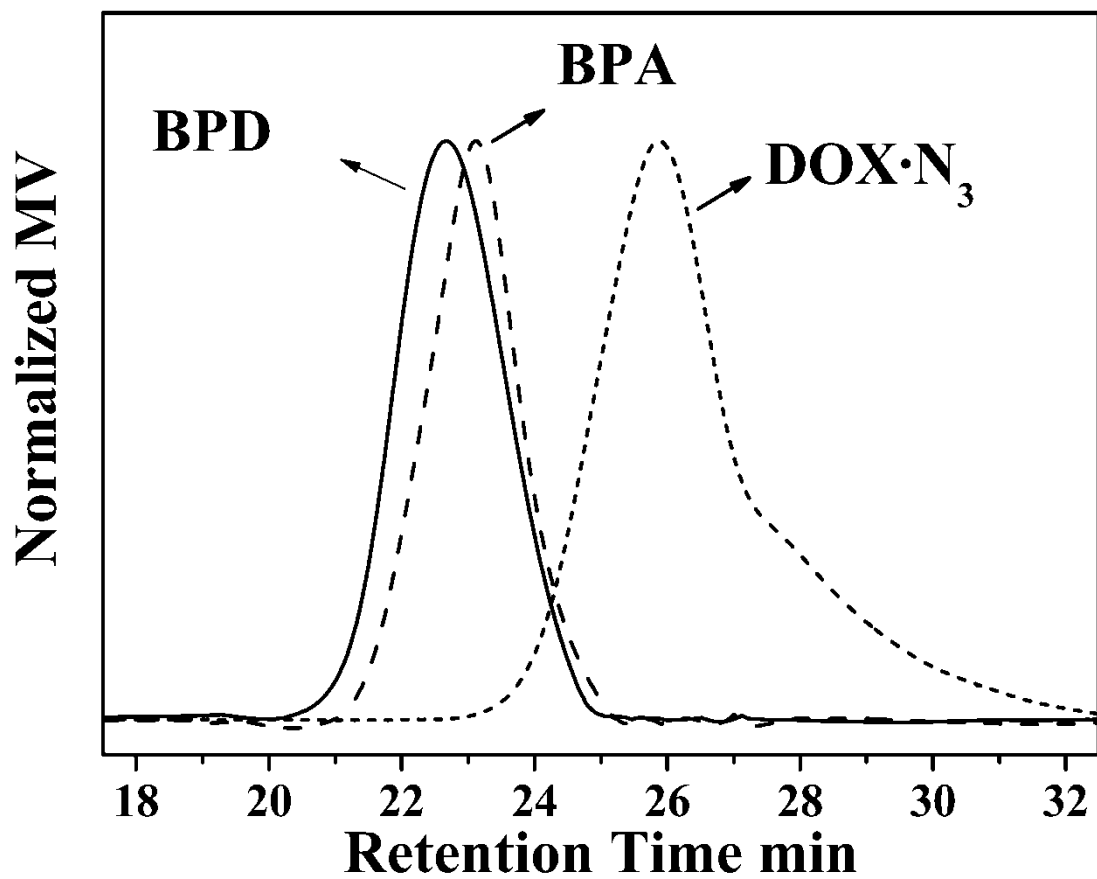


Figure S5. GPC traces of Dox-N<sub>3</sub>, BPA and BPD.

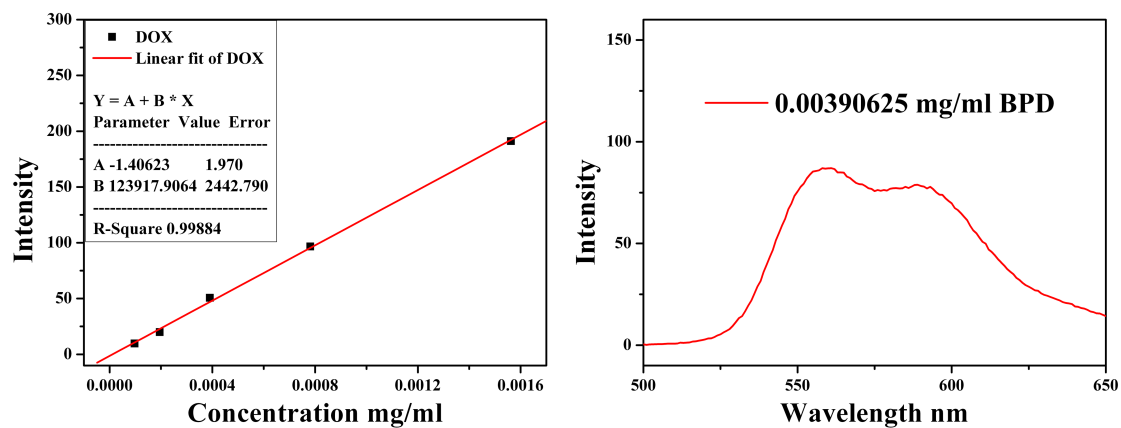


Figure S6. (a) The fluorescence intensity of Dox at emission wavelength of 560 nm, excitation wavelength of 488 nm, and slit width of 5 nm as a function of Dox concentration. (b) The fluorescence spectroscopy of BPD (0.00390625 mg/mL, pre-incubation in 1N HCl for 24 h at rt.) at excitation wavelength of 488 nm, and slit width of 5 nm.

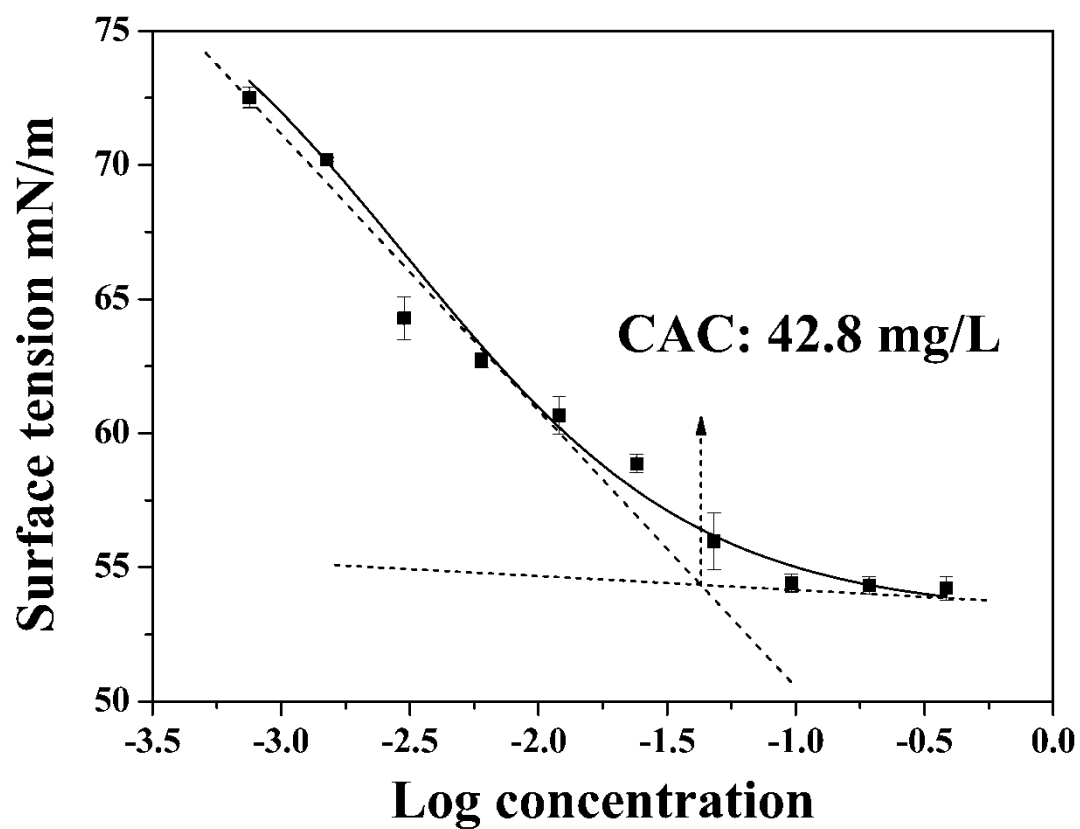


Figure S7. Determination of the critical aggregation concentration of BPD.

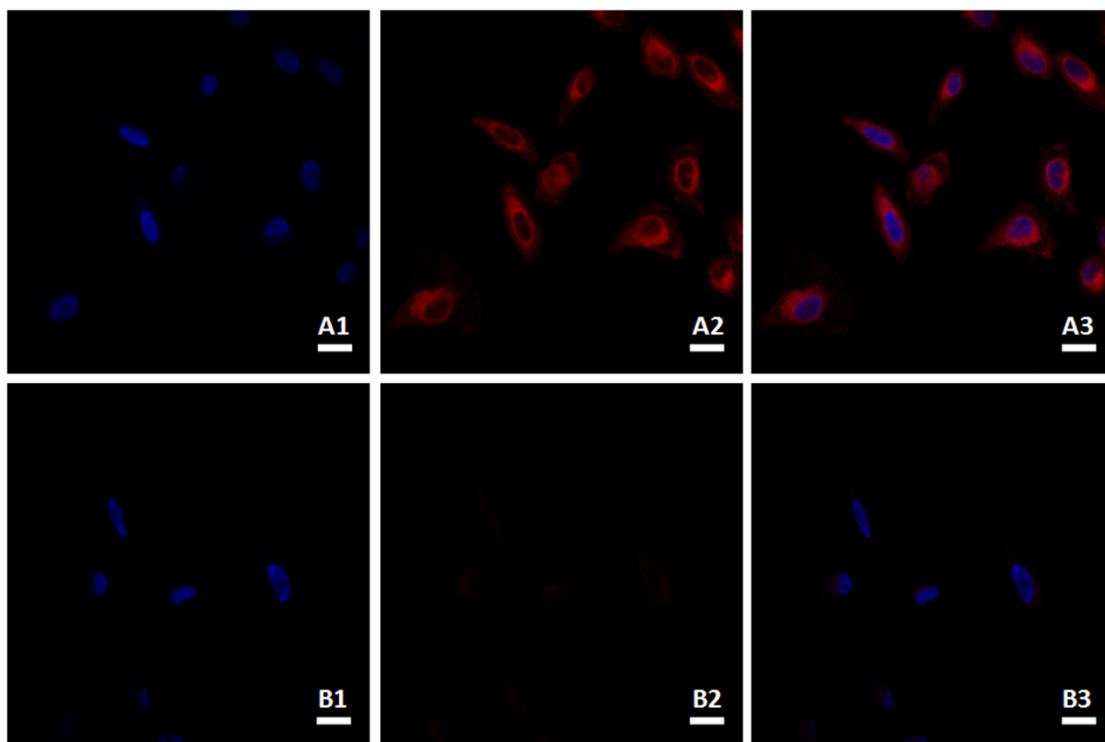


Figure S8. The CLSM images of HeLa cells incubated with BPD (A) and mPD (B) for 1h. 1, 2 and 3 indicated the Hoechst 33258, Dox and merged channels, respectively. Concentration of Dox was 50  $\mu\text{g}/\text{mL}$  and the scale bar is 20  $\mu\text{m}$ .

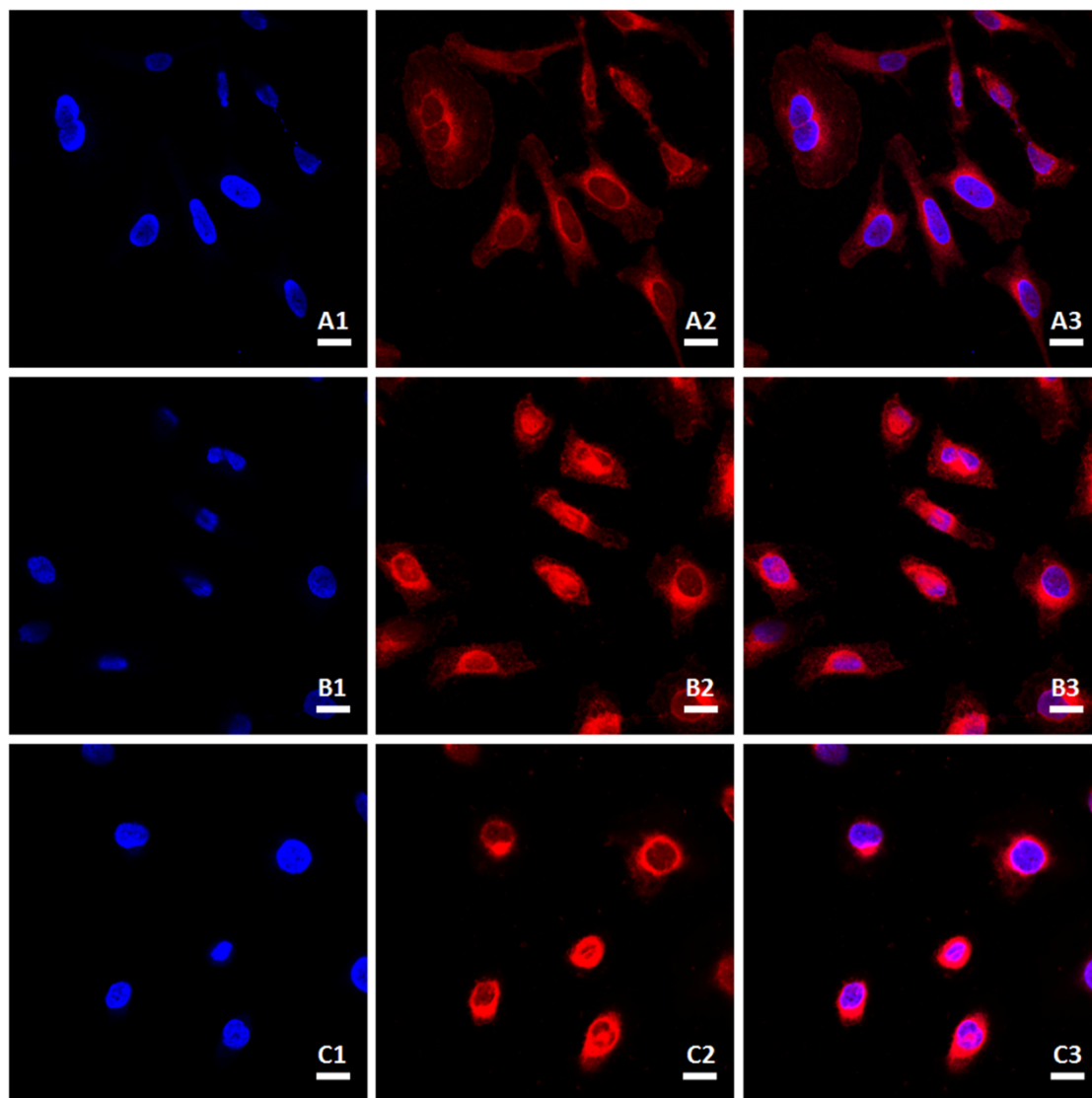


Figure S9. The CLSM images of HeLa cells incubated with BPD/CPT for 4h (A), 12h (B) and 24h (c). 1, 2 and 3 indicated the Hoechst 33258, Dox and merged channels, respectively. Concentrations of Dox and CPT were 50  $\mu\text{g}/\text{mL}$  and 14.5  $\mu\text{g}/\text{mL}$ , respectively, and the scale bar is 20 $\mu\text{m}$ .



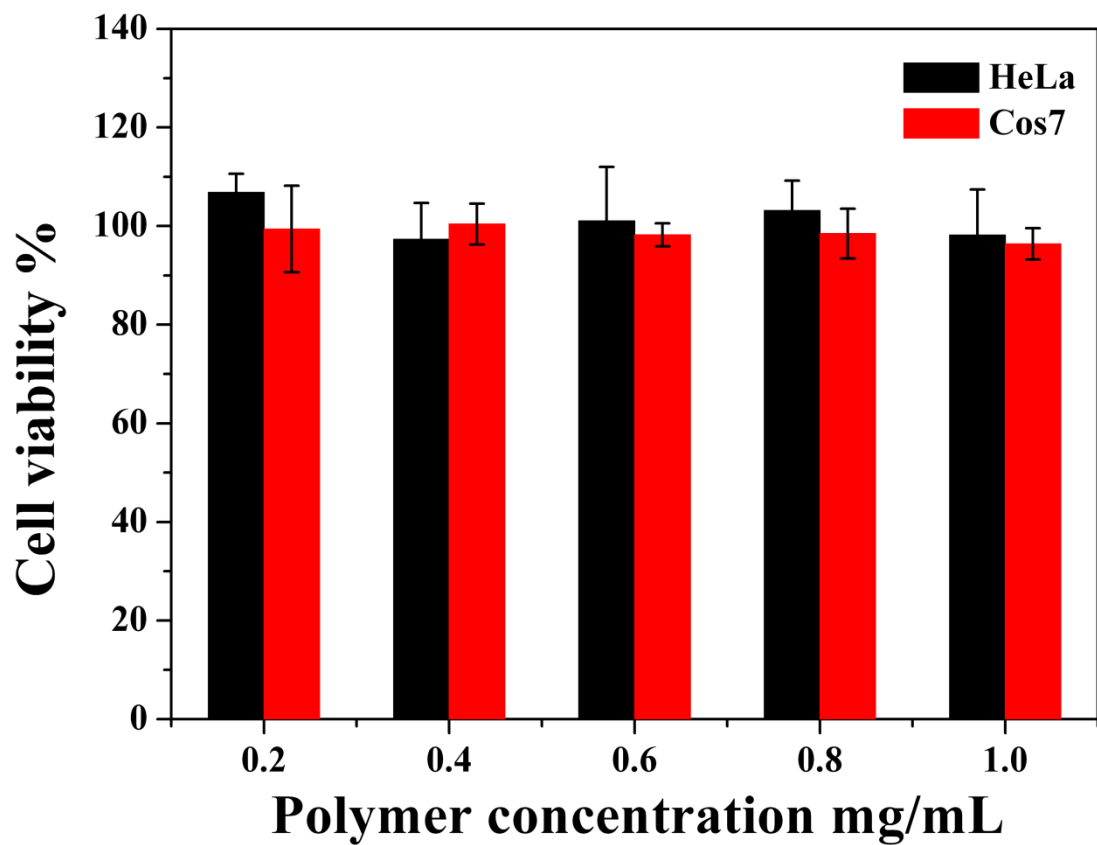


Figure S10. Cytotoxicity of BPA in HeLa cells and Cos7 cells after 48h of incubation.