Supporting Information

Green Tea Catechin-Based Complex Micelles Combined with Doxorubicin to Overcome Cardiotoxicity and Multidrug Resistance

Tangjian Cheng,[1] Jinjian Liu,[2] Jie Ren,[1] Fan Huang,[2] Hanlin Ou,[1] Yuxun Ding,[1] Yumin Zhang,[2] Rujiang Ma[1] Yingli An,[1] Jianfeng Liu,*[,2] and Linqi Shi*[,1, 3]

1. State Key Laboratory of Medicinal Chemical Biology, Key Laboratory of Functional Polymer Materials of Ministry of Education, Institute of Polymer Chemistry, Nankai University, Tianjin 300071, P.R. China.
2. Tianjin Key Laboratory of Radiation Medicine and Molecular Nuclear Medicine, Institute of Radiation Medicine, Chinese Academy of Medical Science & Peking Union Medical College, Tianjin, 300192, P.R. China.
3. Collaborative Innovation Center of Chemical Science and Engineering (Tianjin), Nankai University, Tianjin 300071, P.R. China.

Corresponding authors: Jianfeng Liu, Email: lewis78@163.com. Linqi Shi, Email: shilinqi@nankai.edu.cn

1. Characterization

1H NMR spectra were recorded on a Varian UNITY-plus 400 M NMR spectrometer at room temperature with tetramethylsilane (TMS) as an internal standard. The number-average molecular weight (Mn) and weight-average molecular weight (Mw) were measured by gel permeation chromatography (GPC) at 25 °C with a Waters 1525 chromatograph equipped with a Waters 2414 refractive index detector. GPC measurements were carried out using DMF as eluents with a flow rate of 1.0 mL/min, respectively. Polystyrene and PEG standards were used for calibration. Dynamic light scattering (DLS) experiments at a 90° scatter angle were performed on a laser light scattering spectrometer (BI-200SM) equipped with a digital correlator (BI-9000AT) at 636 nm at required temperature. All samples were obtained by filtering through a 0.45 μm Millipore filter into a clean scintillation vial. Transmission

electron microscopy (TEM) measurements were performed using a Philips T20ST electron microscope at an acceleration voltage of 100 kV. To prepare the TEM samples, the sample solution was dropped onto a carbon-coated copper grid and dried slowly at required temperature. The zeta potential values were measured on a Brookheaven ZetaPALS (Brookheaven Instrument, USA), using phosphate buffer (PB) solution (0.01 M) with a pH range from 5.0 to 7.4 as the background buffer. The instrument utilizes phase analysis light scattering at 37 °C to provide an average over multiple particles. The UV absorption spectra were detected by UV-vis spectrophotometer (Purkinje General, China).

2. Synthesis of polymers

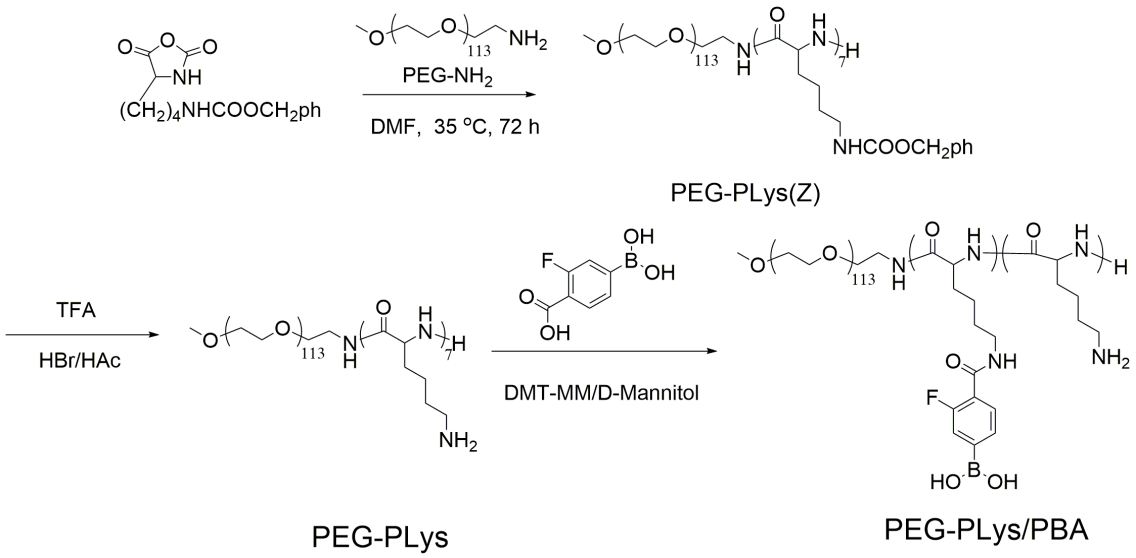

Figure S1. Synthesis of PEG-PLys(Z), PEG-PLys and PEG-PLys/PBA.

PEG-P(Lys-LysDox-LysCy5) was synthesized according to our previous report.[1]

3. Characterization of polymers

3.1 PEG-PLys(Z)

As shown in Fig. S2, the degree of polymerization (Dp) of Lys(Z) was calculated to be 13 by comparing of the peak integration of -OCH_2CH_2- protons of PEG at 3.59 ppm and -OCH2ph protons of PLys(Z) at 5.0 ppm.

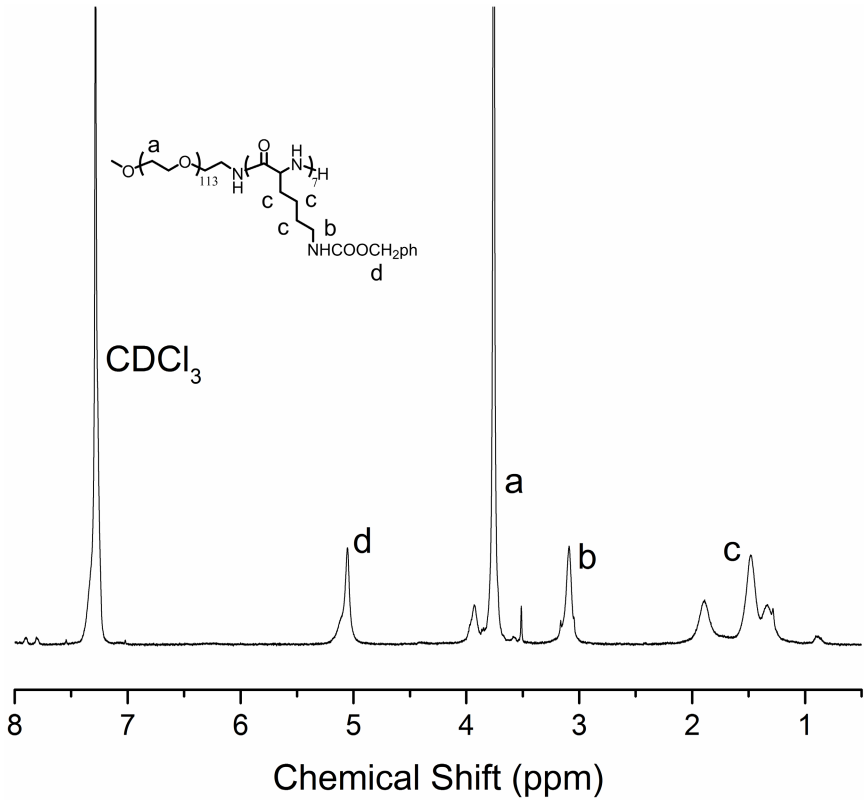

Figure S2. ^{1}H NMR spectra of PEG_{113}-*b*-$PLys(Z)_{13}$ in $CDCl_3$.

3.2 PEG-PLys

The ^{1}H NMR spectrum of PEG-*b*-PLys was shown in Figure S3.

3.3 PEG-PLys/PBA

PEG-PLys/PBA was synthesized by conjugation of FPBA to the pendent amino of PLys using DMT-MM as coupling agent. The ^{1}H NMR spectrum of PEG-*b*-PLys/PBA was shown in Figure S4. The peaks around 7.3 ppm which is the characteristic peaks of the phenylene protons of FPBA indicate that FPBA were conjugated to polymers successfully. The degree of FPBA was estimated to be 6 by calculating the peak intensity ratio of the butylene protons of Lys units (1.2-1.8 ppm) to the phenylene protons of FPBA (7.2-7.5 ppm).

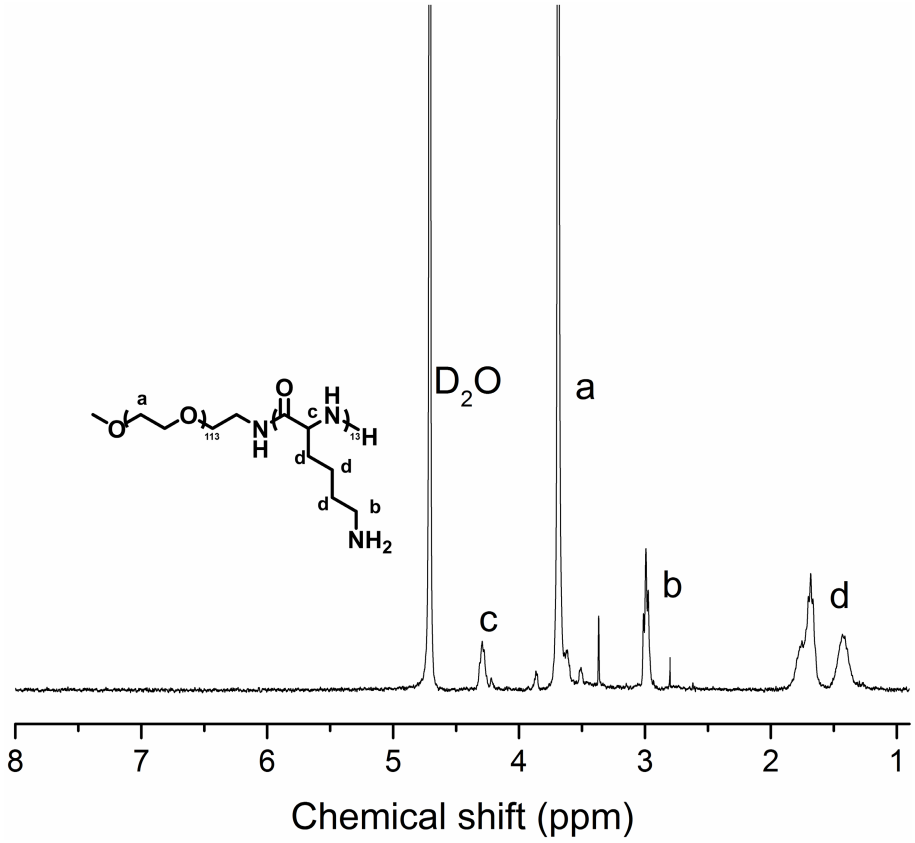

Figure S3. ^{1}H NMR spectra of PEG_{113}-*b*-$PLys_{13}$ in D_2O.

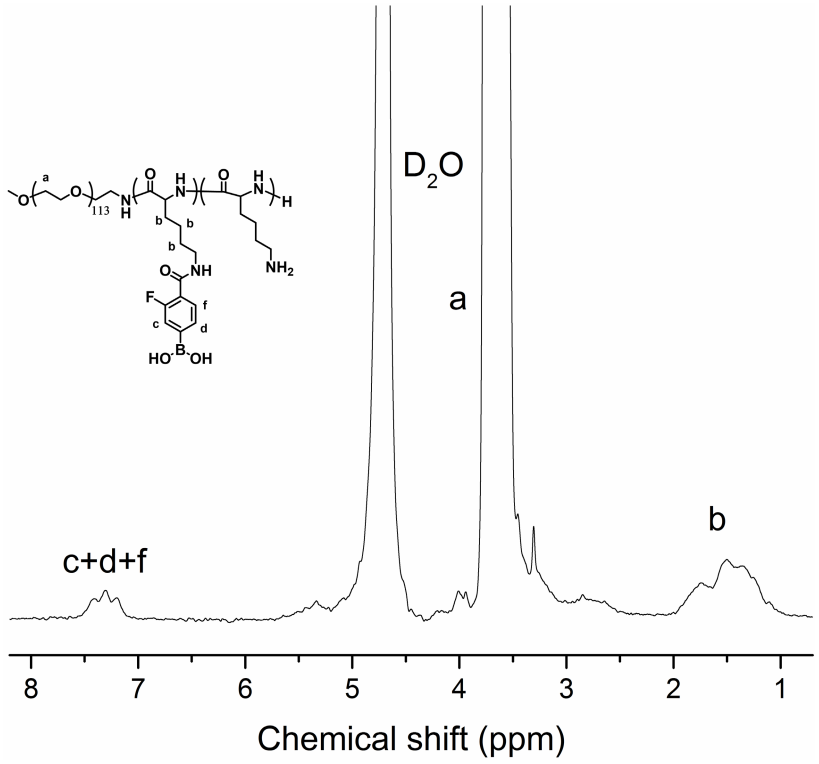

Figure S4. ^{1}H NMR spectra of PEG_{113}-*b*-P(Lys_{13}/PBA_6) in D_2O with addition of 5 mg/mL of D-sorbitol

According to the low scattered light intensity of the polymer solutions (as shown in Figure S5), PEG-PLys/PBA were soluble (at 5 mg/ml) in water because of the low degrees of FPBA modification (46 %) [2]. Therefore, PEG-PLys/PBA could not self-assemble into micelles under physiological environment.

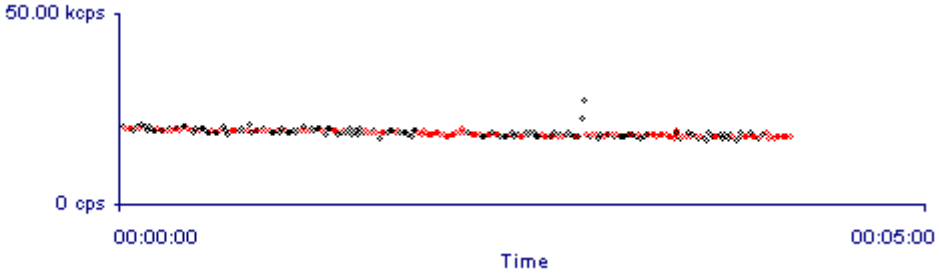

Figure S5. The scattered light intensity of PEG-PLys/PBA solution under physiological condition at 5 mg/ml.

4. Stability of EGCG

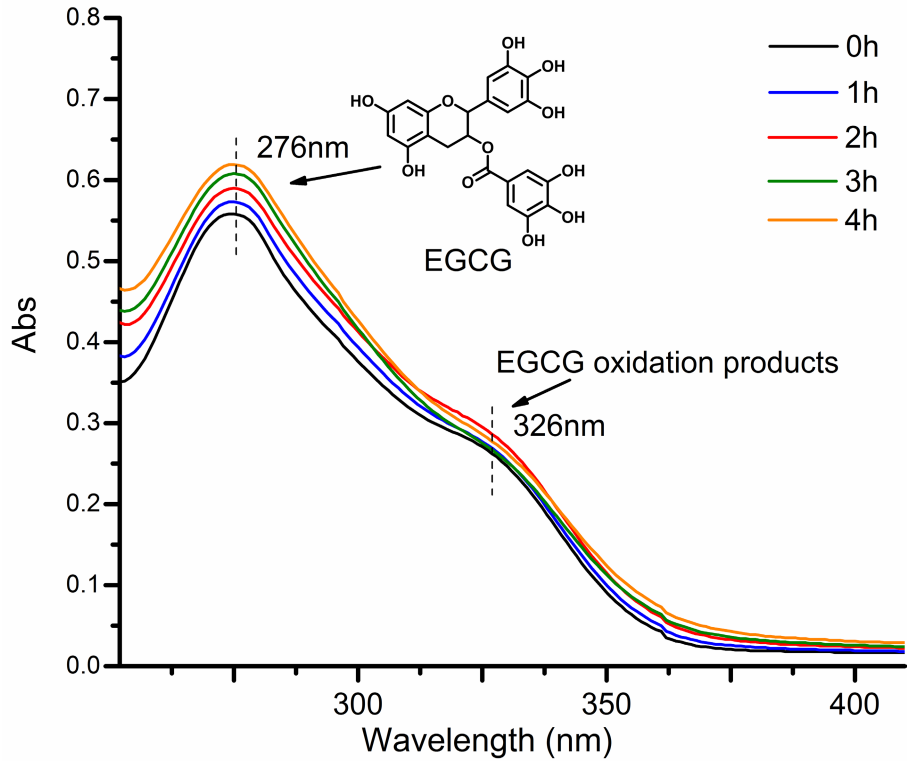

Figure S6. UV-vis spectra of EGCG dissolved in water.

UV-Vis spectrum of EGCG was measured under different pH. As shown in

Figure S7, the absorbance of EGCG at 276 nm decreased slightly and the absorbance of EGCG oxidation products at 326 nm increased with the pH value variation from 5.0 to 7.4. These results implicated that the phenol groups on EGCG have significant effect on the auto-oxidation process of EGCG under different pH condition.

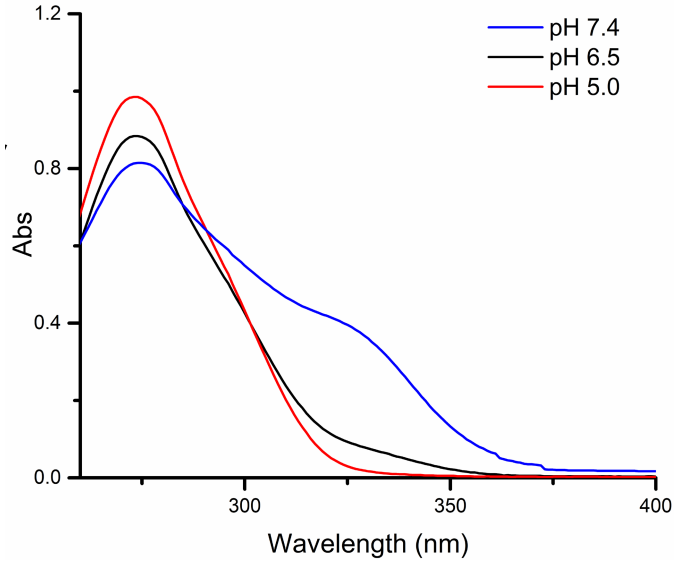

Figure S7. UV-Vis spectrum of EGCG under different pH condition.

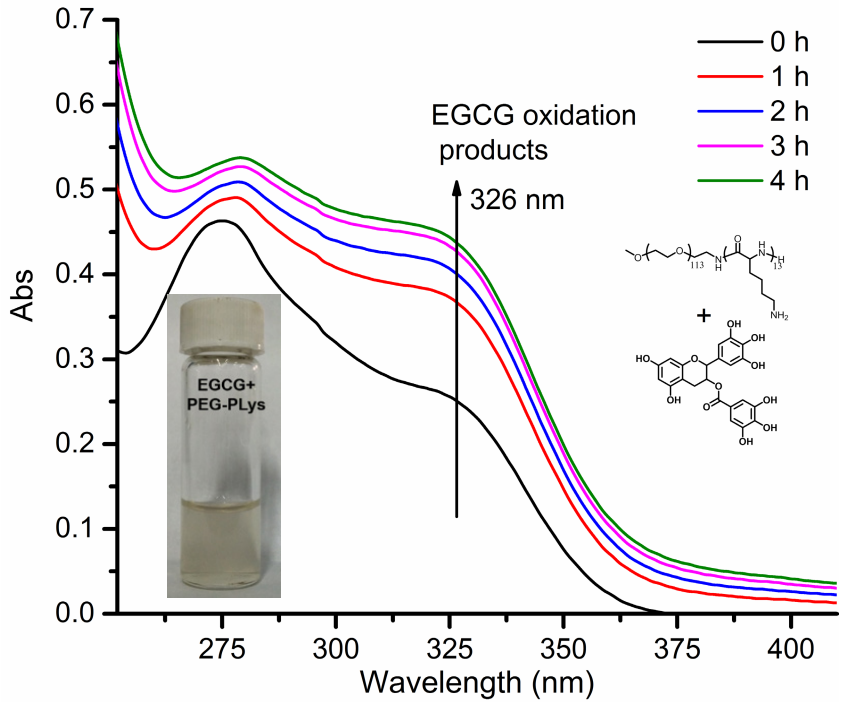

Figure S8. UV-vis spectra of EGCG and PEG-PLys dissolved in water.

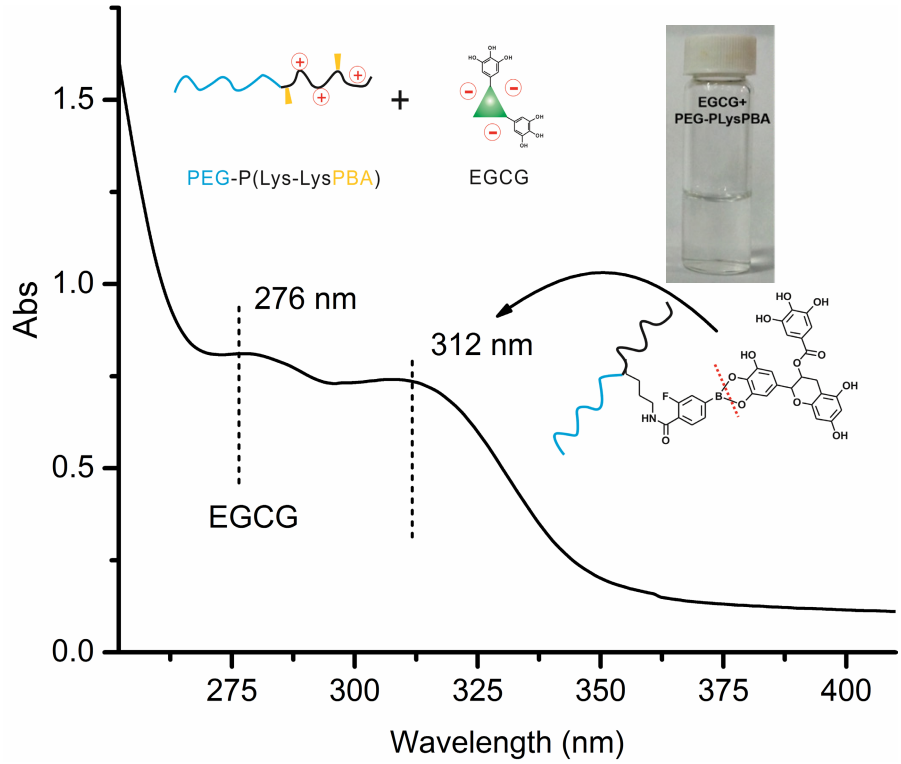

Figure S9. UV-vis spectra of EGCG and PEG-PLys/PBA dissolved in water.

5. Stability of PIC micelles.

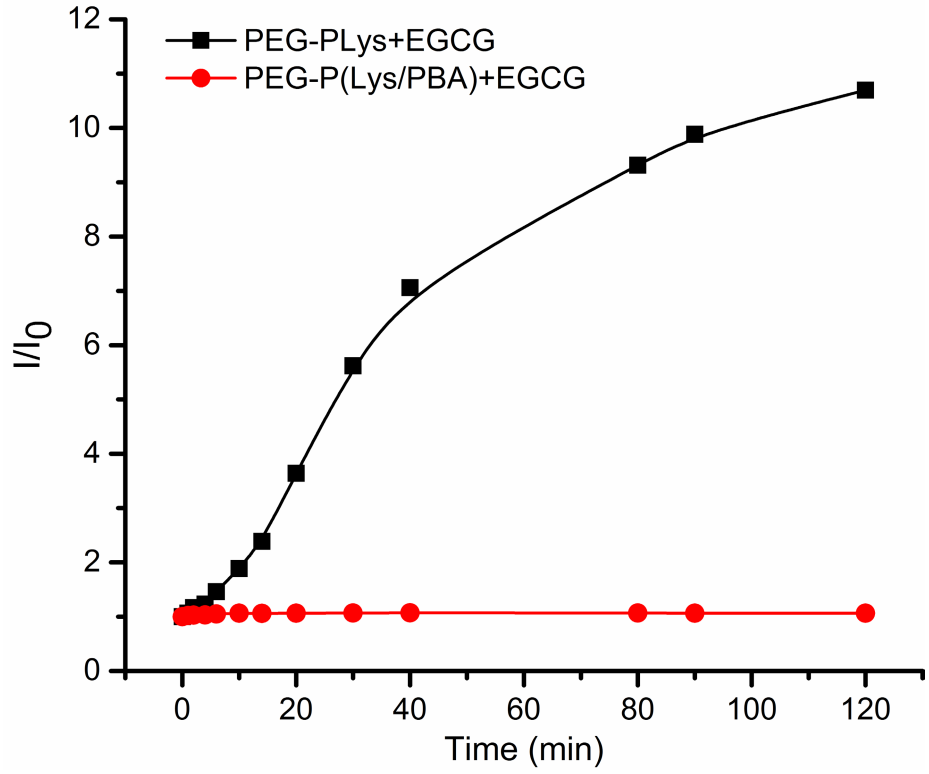

Figure S10. Relative light scattering intensity variation of CLM and NCLM over 2 h in PBS buffer (10 mM) at 37 °C.

Two size distributions were detected, where the larger one around 140 nm should be attributed to CLM while the smaller one around 6 nm could be ascribed to BSA. Over time, these two size distributions did not change a lot. These results indicated that the stability of CLM was excellent in serum.

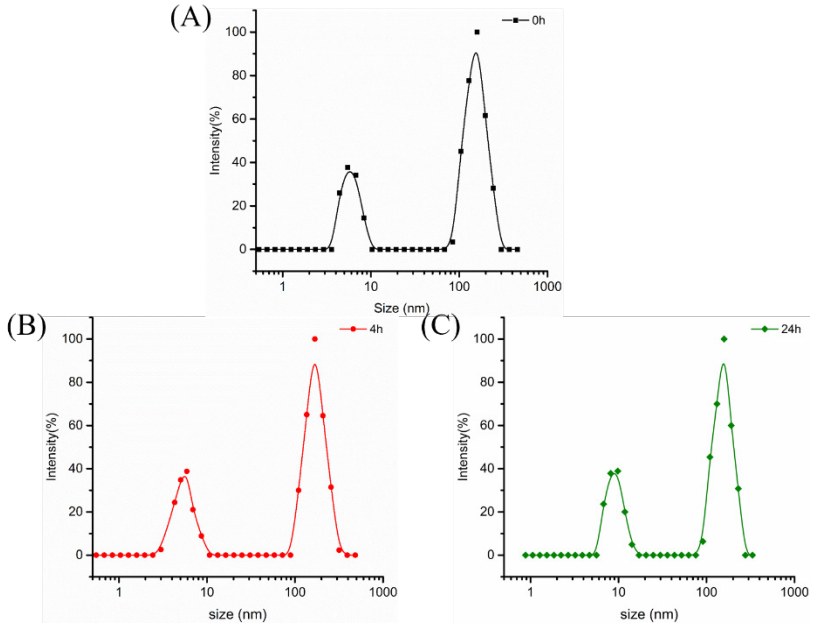

Figure S11. Size distributions of CLM in PBS buffer (pH 7.4, 150 mM NaCl) at 37 ^{o}C in the presence of 45 g/L BSA at different time points, 0 h (A), 4 h (B) and 24 h (C).

6. Cytotoxicity against MCF-7 cells

EGCG based CLM exhibited higher toxicity against MCF-7 than NP because of EGCG inhibition of MCF-7 cells growth as shown in Figure S12, which is consistent with previous reports [3]. Because of EGCG inhibition of MCF-7 cells growth, CLM-DOX exhibited higher toxicity than NP-DOX as shown in Figure S12.

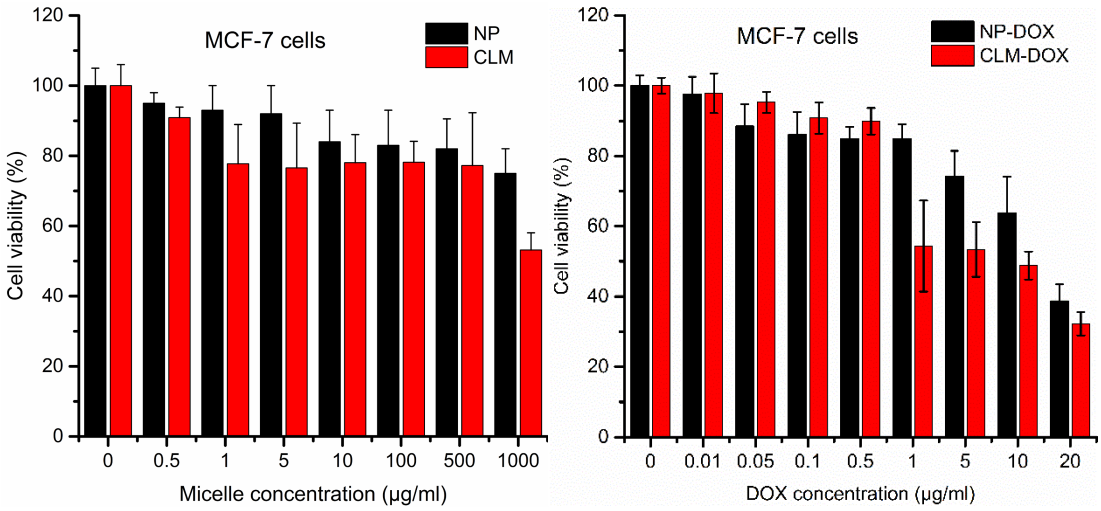

Figure S12. Cytotoxicity of different micelle against human breast cancer cells MCF-7 cells. Dose-response curves of DOX in CLM-DOX and NP-DOX against MCF-7. (Mean ±standard deviation, n=3)

7. Flow cytometry

For quantitative analysis of cellular uptake, H9C2 cells were seeded into 96-well plates at a density of 0.6×104 cells per well in 100 μL RPMI-1640 medium/PBS. After an incubation of 24 hours, the culture medium of each well was replaced with 500 μL of fresh medium, then PBS (as control), free DOX and the DOX-loaded micelles (CLM-DOX and NP-DOX) were added to each well. After 2 hours further incubation, the culture medium was removed, cells were washed three times with 500 μL PBS buffer and detached by 0.02% (w/v) EDTA and 0.25% (w/v) trypsin solution, and then dispersed in 0.25 mL of PBS for flow cytometric measurement. Cells treated with PBS were used as control.

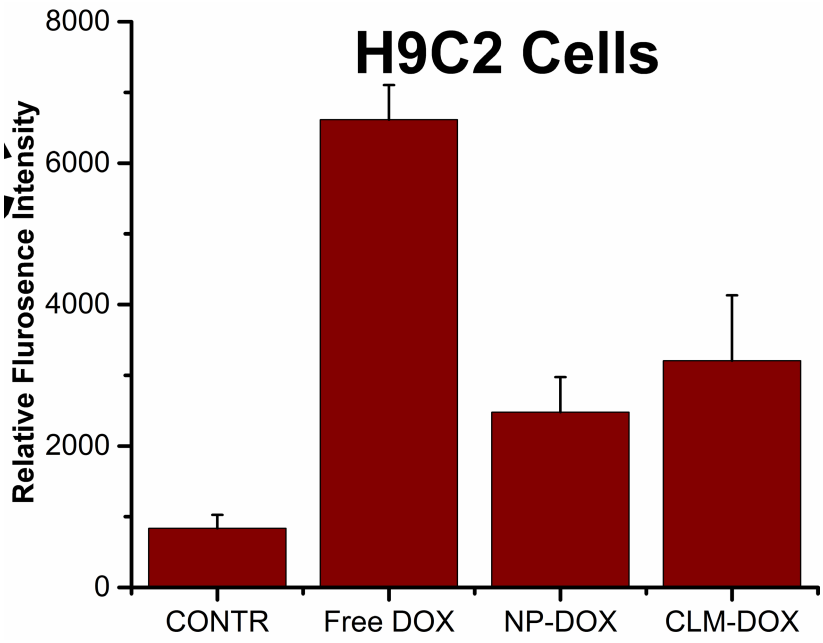

Figure S13. The flow cytometry analysis 4 h after incubation in H9C2 cells.

For quantitative analysis of cellular uptake, MCF-7/ADR cells were seeded into 96-well plates at a density of 0.6×10^4 cells per well in 100 μL RPMI-1640 medium/PBS. After an incubation of 24 hours, the culture medium of each well was replaced with 500 μL of fresh medium, then PBS (as control), free DOX and the DOX-loaded micelles (CLM-DOX and NP-DOX) were added to each well. After 4 hours further incubation, the culture medium was removed, cells were washed three times with 500 μL PBS buffer and detached by 0.02% (w/v) EDTA and 0.25% (w/v) trypsin solution, and then dispersed in 0.25 mL of PBS for flow cytometric measurement. Cells treated with PBS were used as control.

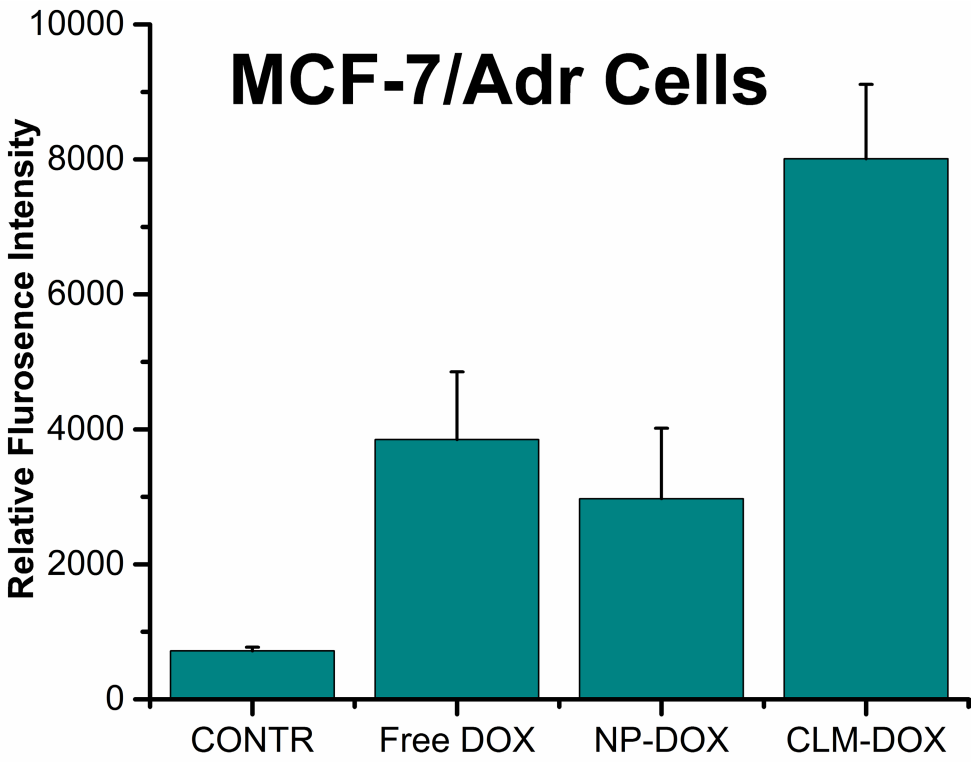

Figure S14. The flow cytometry analysis 4 h after incubation in MCF-7/Adr cells.

8. ROS generation in cancer cells

ROS generation in cancer cells incubated with different solution (PBS, free DOX, NP-DOX and CLM-DOX) were measured using DCFH-DA as the ROS probe. As shown in Figure S15, cells incubated with CLM-DOX exhibited lowest intracellular ROS generation which was similar with the result in H9C2 cells. It indicated that ROS generated by DOX could be eliminated by EGCG in cancer cells.

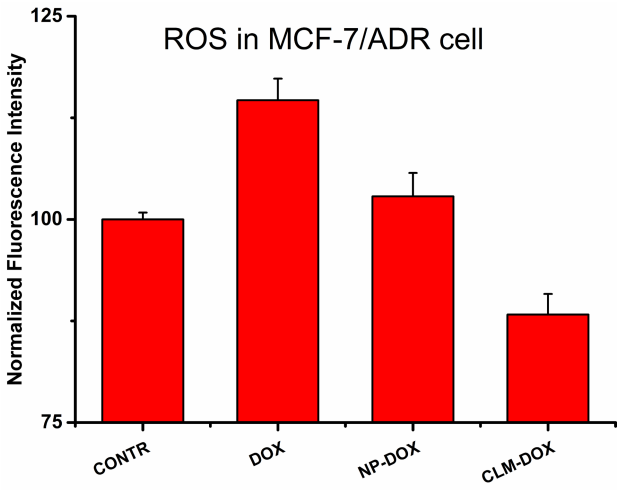

Figure S15. ROS content in MCF-7/ADR cells treated with PBS, free DOX, NP-DOX and EGCG based CLM-DOX after 2 h incubation.

Reference

1. Cheng T, Ma R, Zhang Y, Ding Y, Liu J, Ou H, et al. A surface-adaptive nanocarrier to prolong circulation time and enhance cellular uptake. Chem Commun. 2015; 51: 14985-8.

2. Naito M, Ishii T, Matsumoto A, Miyata K, Miyahara Y, Kataoka K. A Phenylboronate-Functionalized Polyion Complex Micelle for ATP-Triggered Release of siRNA. Angew Chem Int Ed. 2012; 51: 10751-5.

3. Zhu A, Wang X, Guo Z. Study of tea polyphenol as a reversal agent for carcinoma cell lines' multidrug resistance (study of TP as a MDR reversal agent). Nucl Med Biol. 2001; 28: 735-40.