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Supplementary Information

Lipid-polymer hybrid nanoparticles for synergistic drug delivery to overcome cancer drug resistance

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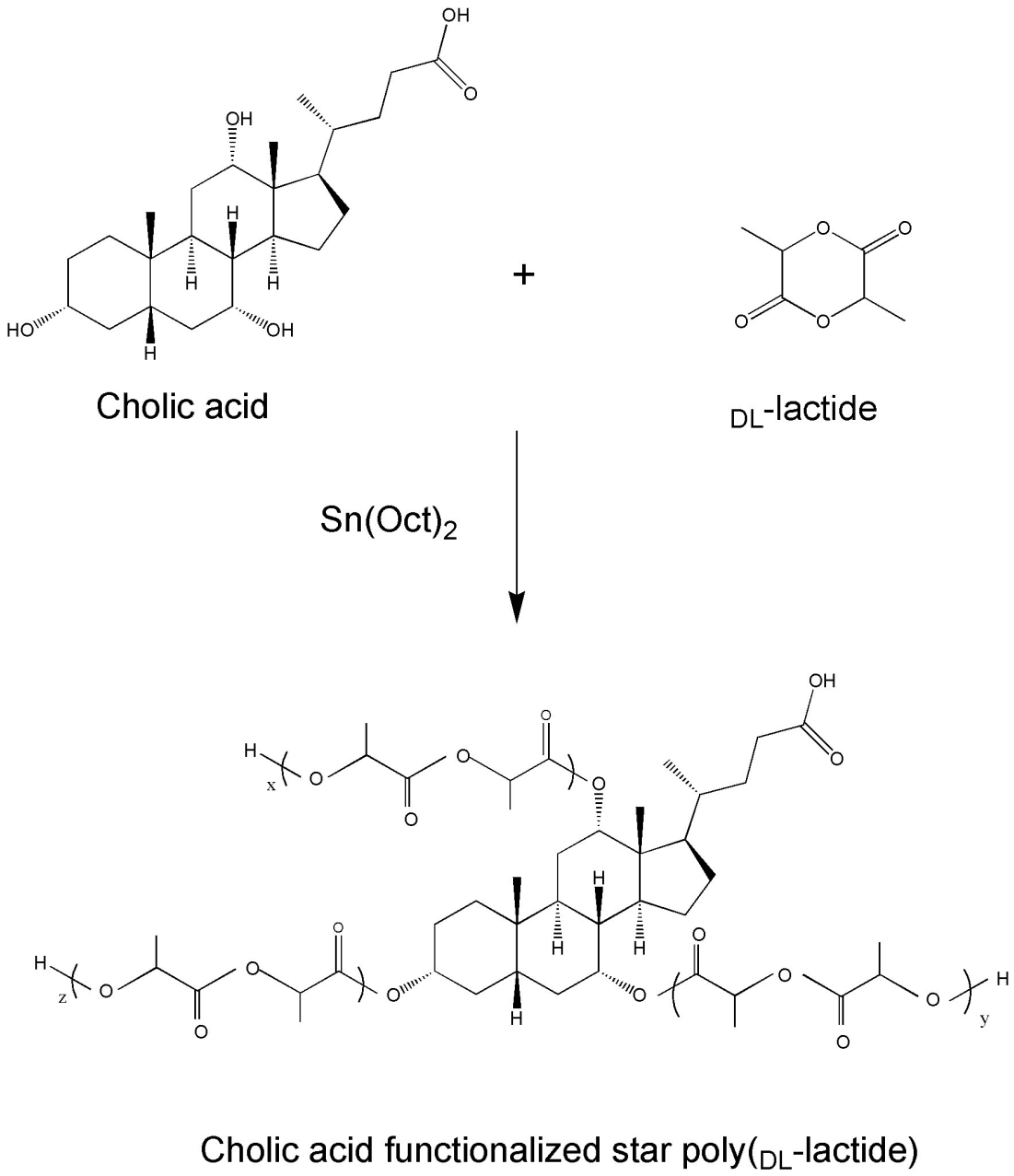
Synthesis of cholic acid functionalized star poly($_{DL}$-lactide)

Cholic acid functionalized star poly($_{DL}$-lactide) was synthesized according to a reported procedure.[25] Briefly, a mixture of cholic acid, $_{DL}$-lactide and catalyst solution ($Sn(Oct)_2$ in toluene) was placed in a thoroughly dried silanized glass flask with a magnetic stirring bar. The molar ratio of cholic acid to $_{DL}$-lactide was 1:15. The catalyst $Sn(Oct)_2$ content in the reaction system was 0.1 mol%. After the flask was evacuated, purged with N_2 and sealed, the reaction system was immersed in an oil bath preheated to 200 ºC for 5 min to allow cholic acid to melt quickly, and then moved to another oil bath at 150 ºC to carry out the polymerization for 12 h. After the polymerization, the product was dissolved in tetrahydrofuran (THF) and precipitated in ethanol/water (3/1 v/v). The molecular weight (Mw) of cholic acid functionalized star poly($_{DL}$-lactide) determined by combined size-exclusion chromatography and multiangle laser light scattering analysis (SEC-MALLS) was 6×10^3 g/mol.



Scheme S1. Synthesis of cholic acid functionalized star poly($_{DL}$-lactide).

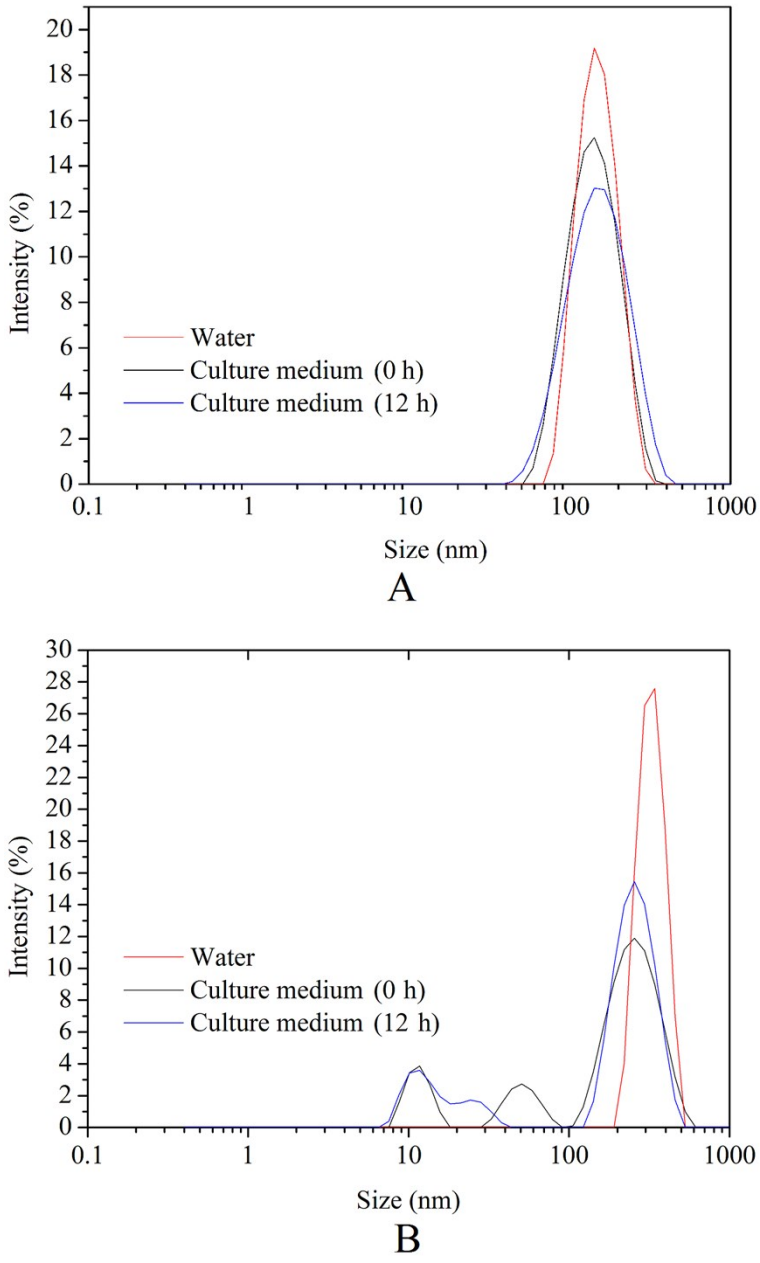


Fig. S1. The size distributions of (A) LPNP (with DSPE-PEG2000) and (B) phosphatidylcholine-shell and polymer-core nanoparticles (without DSPE-PEG2000) after different storage times.

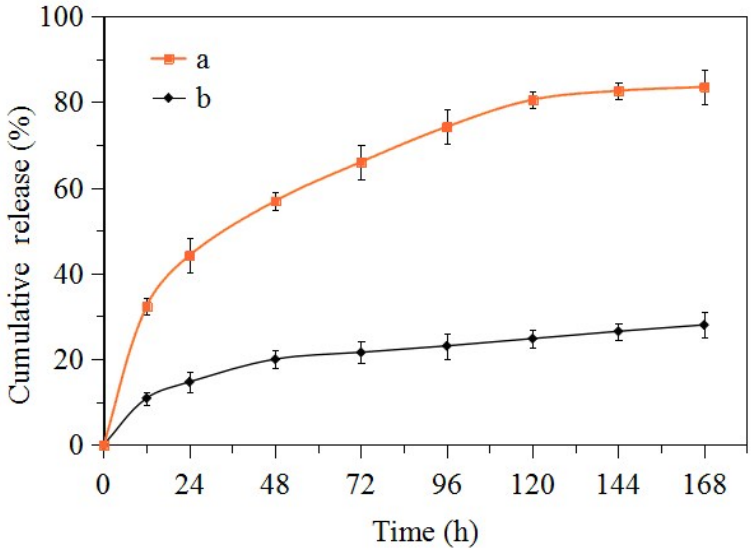


Fig. S2. *In vitro* PTX release profiles of (a) LPNP with the core of cholic acid functionalized star poly($_{DL}$-lactide) and (b) lipid-shell and polymer-core nanoparticles with the core of linear poly($_{DL}$-lactide) in PBS (pH 7.4) containing 1 wt% DMSO.

In this study, we also prepared the lipid-shell and polymer-core nanoparticles with the core of linear poly($_{DL}$-lactide). As shown in Fig. S2, the nanoparticles with the core of linear poly($_{DL}$-lactide) exhibit a much slower release rate compared with the nanoparticles with the core of cholic acid functionalized star poly($_{DL}$-lactide). This result implies that the nanoparticles with the core of cholic acid functionalized star poly($_{DL}$-lactide) have a better drug release performance since a fast intracellular drug release is favorable after the drug loaded nanoparticles are uptaken by cells.

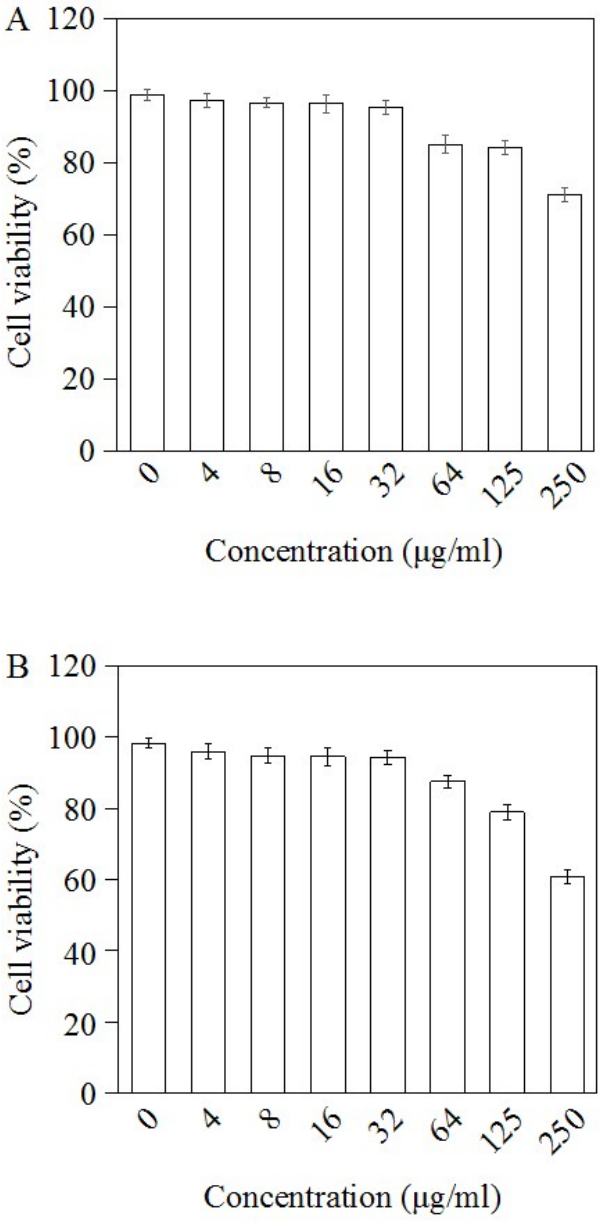


Fig. S3. Cell viability of (A) MCF-7/ADR cells and (B) HeLa cells after being treated by blank LPNP for 48 h.

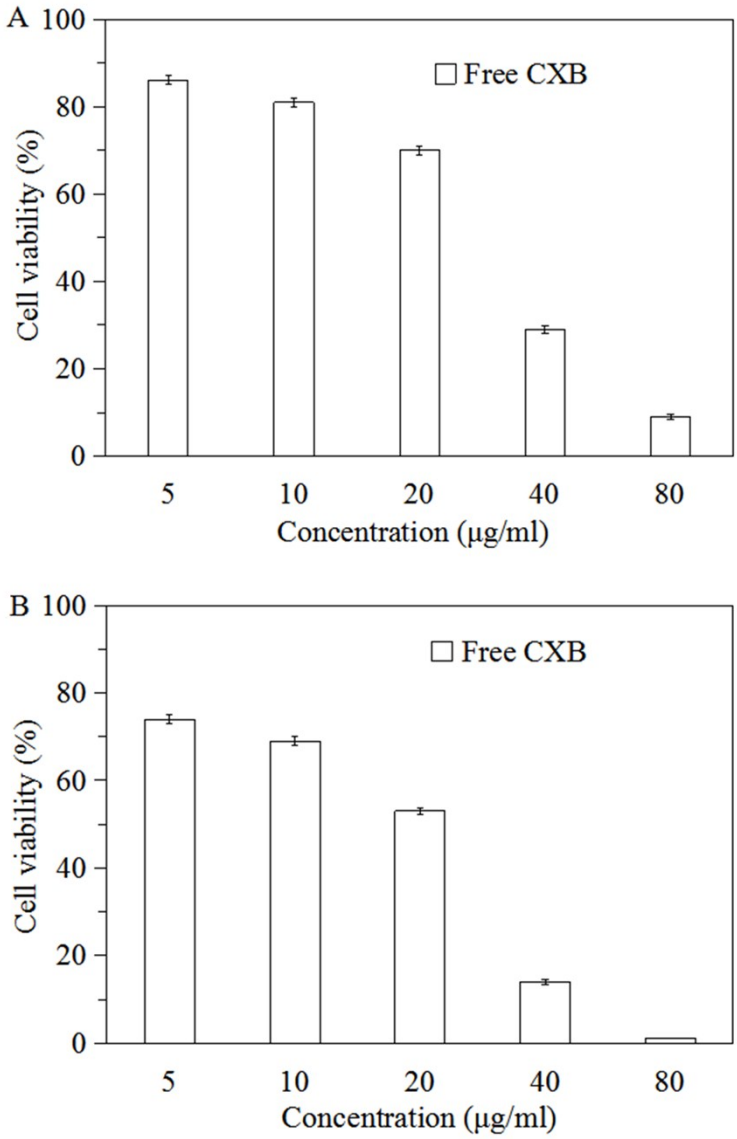


Fig. S4. Cell viability of (A) MCF-7/ADR cells and (B) HeLa cells after being treated by free CXB for 48 h.

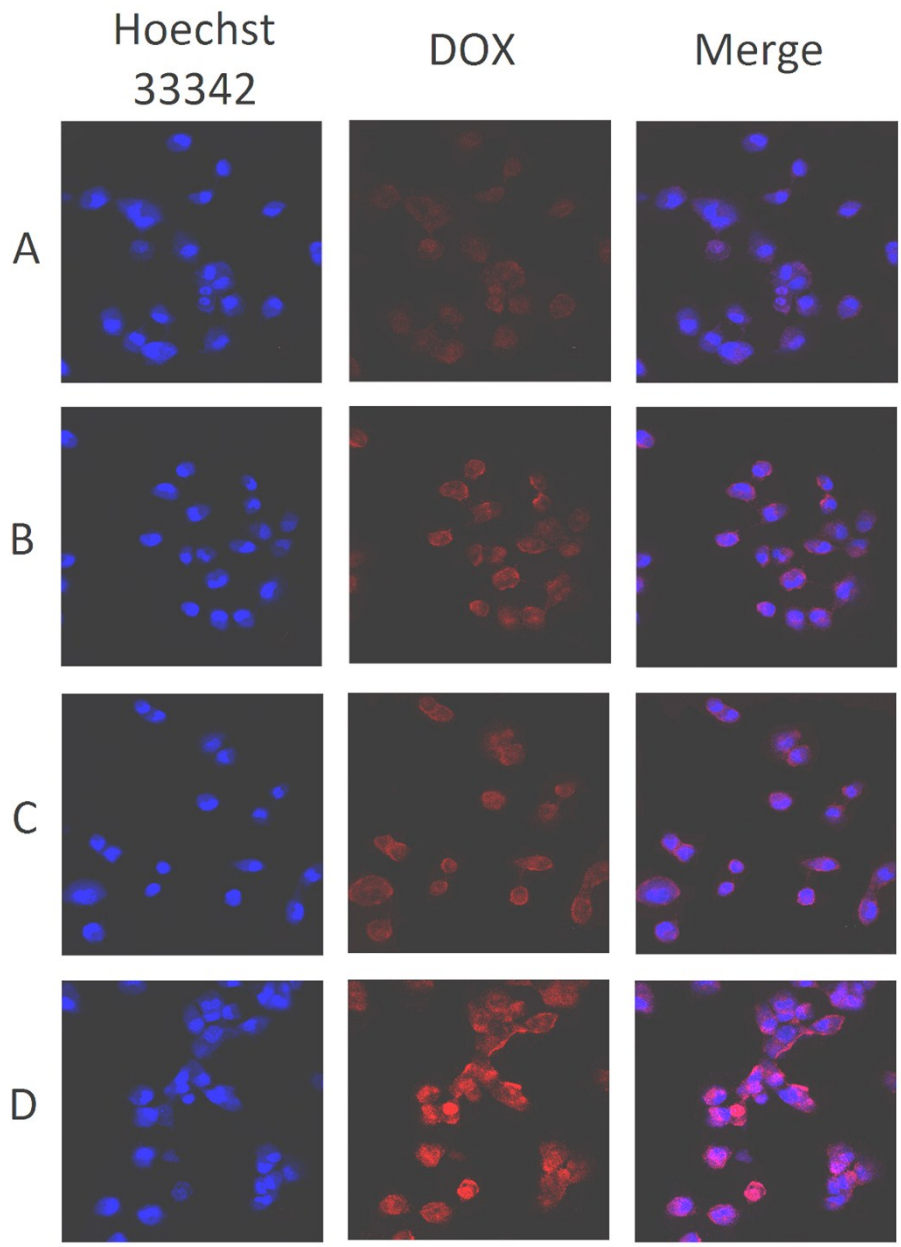


Fig. S5. Confocal images of MCF-7/ADR cells after different treatments for 4 h. (A) free DOX, (B) DOX@LNP, (C) DOX@LPNP, (D) DOX/CXB@LPNP.

MCF-7/ADR cells (1×10^5) were seeded in a 35 mm glass-bottomed culture dish. After 24 h, the culture medium was removed and the cells were co-incubated with a particular agent with a DOX concentration of 10 μg/ml. After 4 h, the medium was

removed and the cells were washed with 1 ml of PBS three times. After that, the nuclei were stained by Hoechst 33342. The cells was washed thrice by PBS and observed by a confocal laser scanning microscope (Nikon Ni-E C2+).

Doxorubicin hydrochloride (DOX) which emits red fluorescence instead of PTX was loaded in LPNP, and the MCF-7/ADR cells treated by DOX loaded LPNP were observed by CLSM. As shown in Fig. S5, the MCF-7/ADR cells treated by DOX@LNP and DOX@LPNP exhibit obviously higher red fluorescences as compared with the cells treated by free DOX. This is due to the “stealth” endocytosis of drug loaded nanoparticles, i.e., the nanocarriers prevent DOX molecules from being recognized by overexpressed P-gp. As a result, the internalized nanoparticles resulted in an increased intracellular DOX concentration. As expected, DOX/CXB@LPNP results in the highest intracellular DOX concentration, which is due to the effective down-regulation of P-gp by CXB. The observation of CLSM is in good agreement with the cytotoxicity evaluation.