Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

SUPPLEMENTARY INFORMATION

For

Phycocyanin-based nanocarrier as a new nanoplatform for efficient overcoming of cancer drug resistance

Results

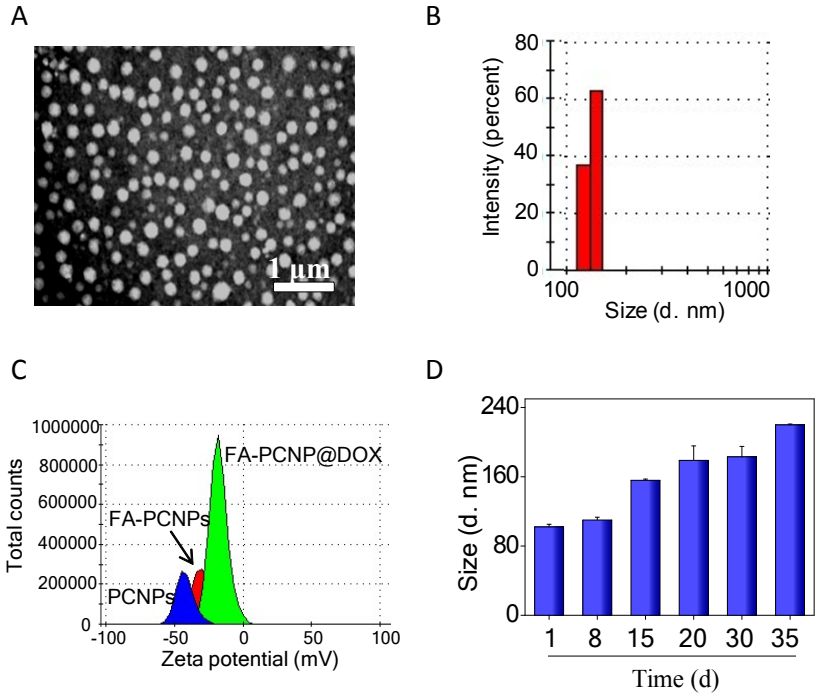

Figure S1. (A) TEM image of PCNPs. (B) Size distribution of PCNPs. (C) Zeta potential of PCNPs, FA-PCNPs and FA-PCNP@DOX. (D) Size variation of PCNPs in aqueous solution monitored for 35 days. Values expressed were means ± SD of 3 independent experiments.

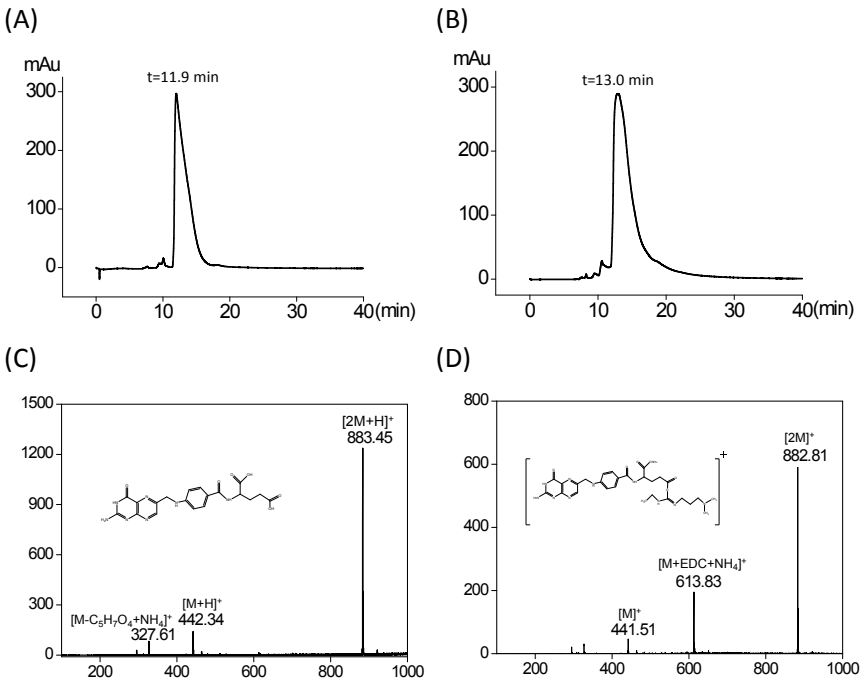

Figure S2. HPLC spectra of compound (A) FA and (B) FA-EDC. The ESI-MS spectra of compound (C) FA and (D) FA-EDC.

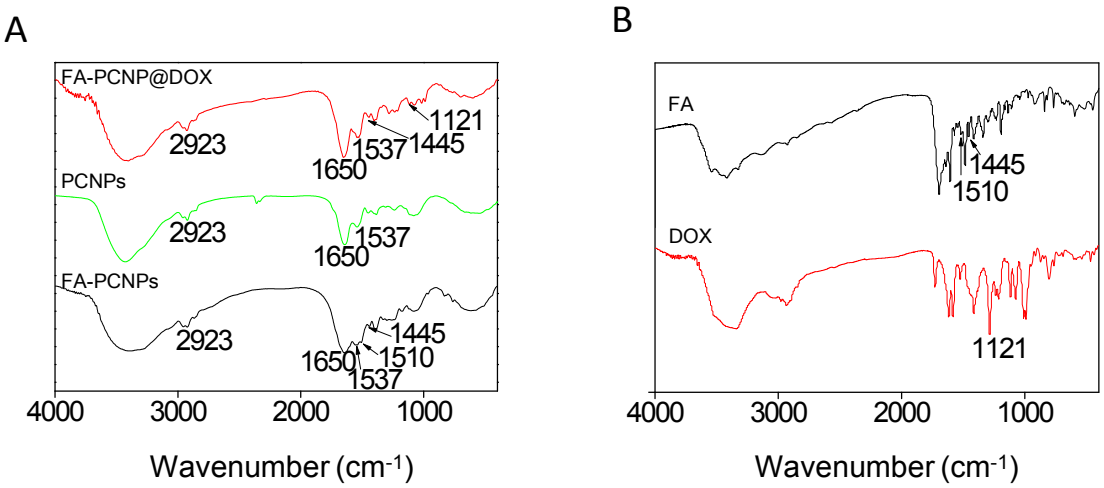

Figure S3. (A) FT-IR spectra of PCNPs, FA-PCNPs and FA-PCNP@DOX. (B) FT-IR spectra of FA and DOX.

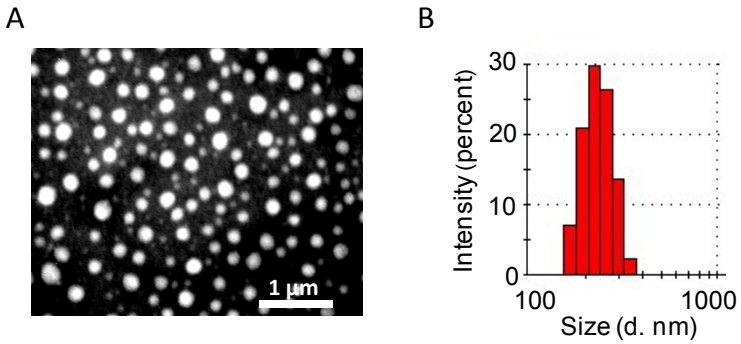

Figure S4. (A) TEM image of FA-PCNP@DOX. (B) Size distribution of FA-PCNP@DOX.

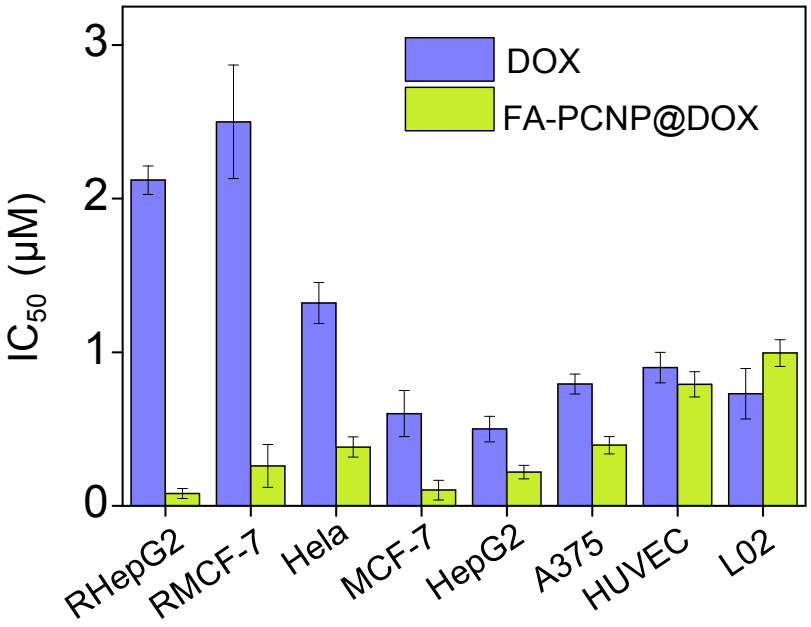

Figure S5. The cytotoxicities of FA-PCNP@DOX or DOX towards RHepG2, RMCF-7, Hela, MCF-7, HepG2, A375, HUVEC and L02 cells for 36 h. Values expressed were means ± SD of 3 independent experiments.

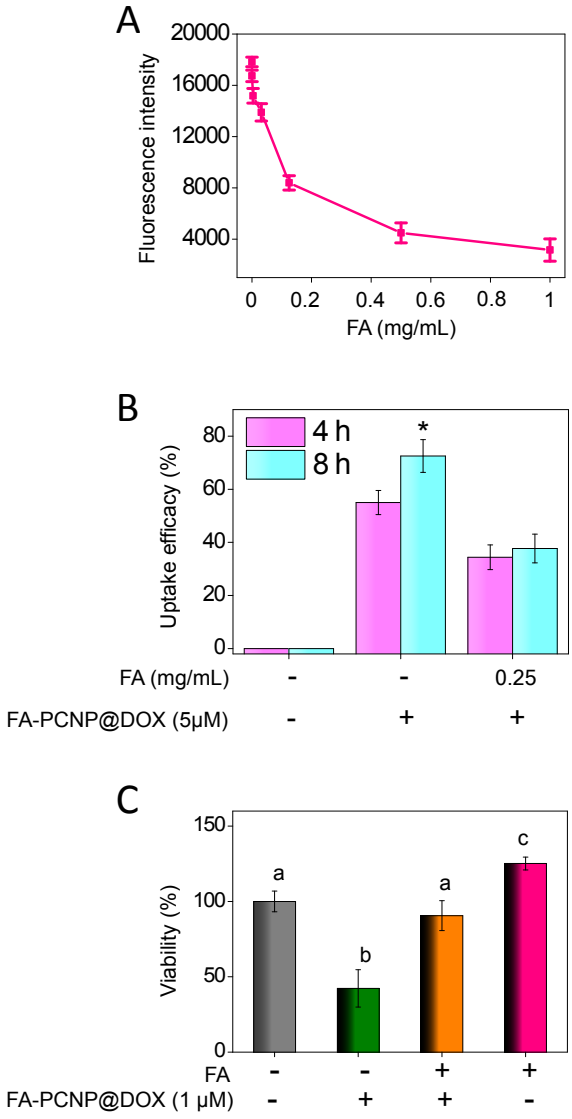

Figure S6. (A) The fluorescence intensity of FA-PCNP@DOX in RHepG2 cells after blocked with excess amount of FA. The cells pretreated with different concentrations of FA for 1 h were subjected to 5 μM FA-PCNP@DOX treatment for 3 h. The fluorescence of FA-PCNP@DOX in the cells was determined with the excitation and emission wavelength at 485 nm and 590 nm respectively. (B) The uptake efficiency of FA-PCNP@DOX in RHepG2 after treated with excess FA 0.25 mg/mL. 5 μM FA-PCNP@DOX was initially fed to the RHepG2 cells. At the time point of 4 h and 8 h, the uptake efficiency of FA-PCNP@DOX was measured. * indicated the statistical difference of uptake efficacy in the time point of 4 h and 8 h at $P < 0.05$ level. (C) The cytotoxicity of FA-PCNP@DOX in RHepG2 cells after treated with excess FA (0.25 mg/mL). The cell viability was measured after 24 h of FA-PCNP@DOX treatment. Different letters represented significantly different means in three or more groups ($P < 0.05$, Tukey's test, one-way ANOVA). Values expressed were means ± SD of 3 independent experiments.

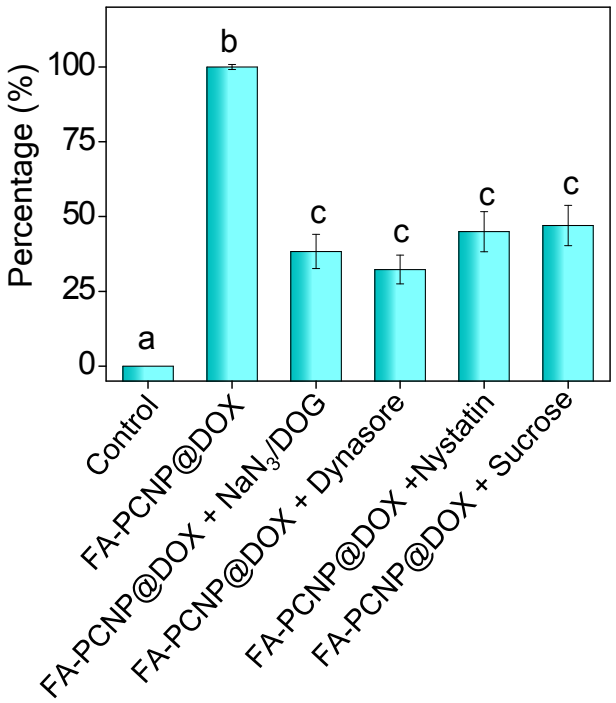

Figure S7. Intracellular uptake of FA-PCNP@DOX in RHepG2 cells with the pretreatment of different endocytosis inhibitors. Different letters represented significantly different means in three or more groups ($P < 0.05$, Tukey's test, one-way ANOVA). Values expressed were means ± SD of 3 independent experiments.

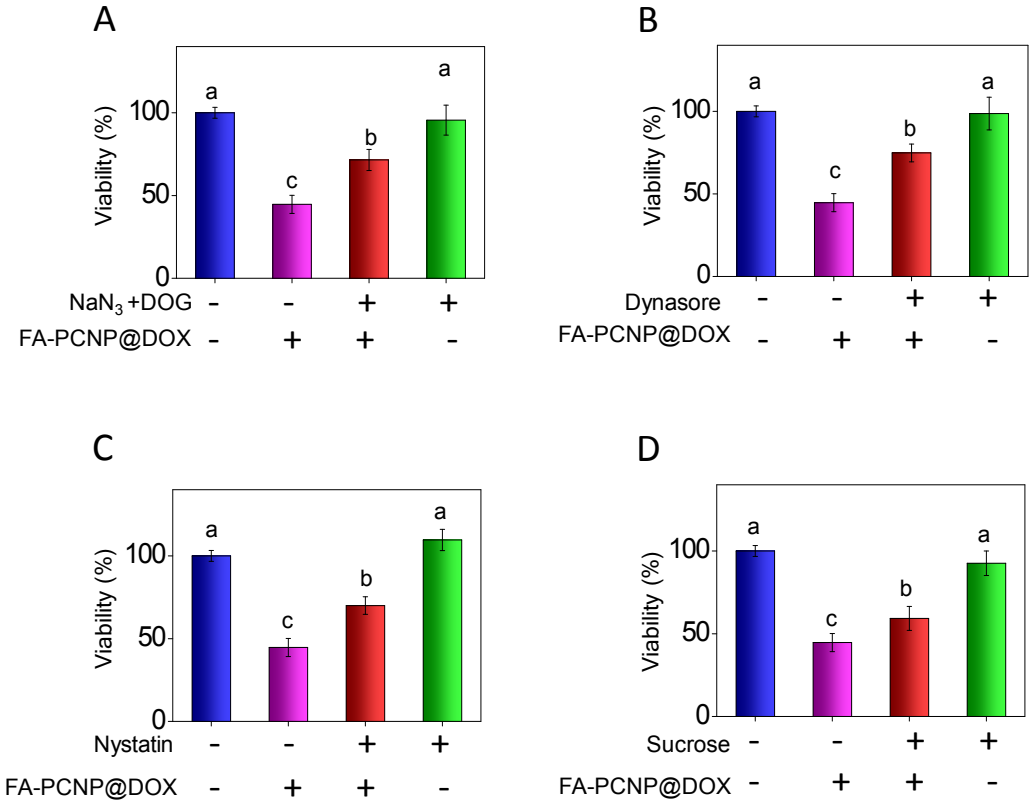

Figure S8. The pretreatment of NaN_3/DOG, dynasore, nystatin and sucrose decreased the cytotoxicity of FA-PCNP@DOX (1 μM) in RHepG2 cells at 24 h of treatment. Values expressed were means ± SD of 3 independent experiments. Different letters represented significantly different means in three or more groups ($P < 0.05$, Tukey's test, one-way ANOVA).

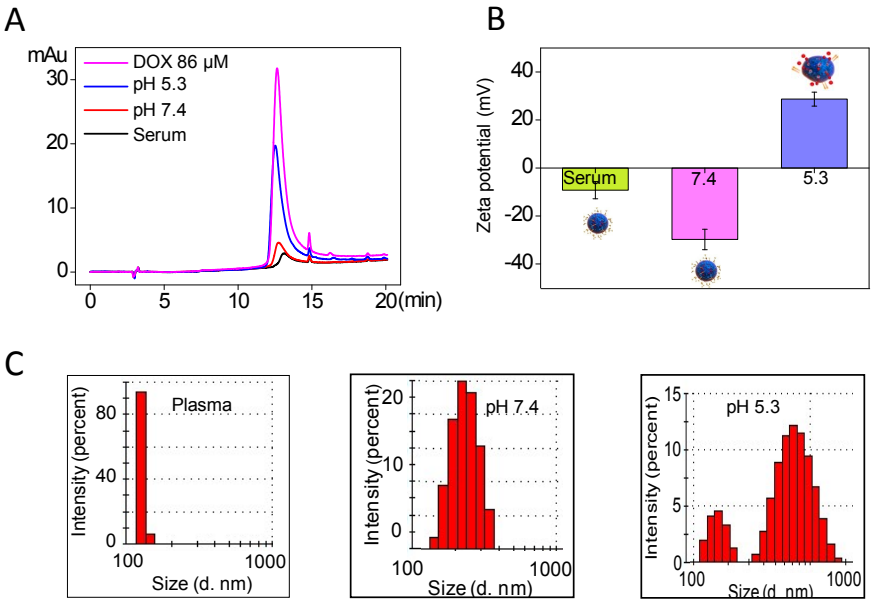

Figure S9. (A) The HPLC spectra of DOX released from FA-PCNP@DOX at pH 7.4, pH 5.3 or in serum. The spectrum of DOX with the concentration of 86 μM recorded the standard curve of DOX. The zeta potential (B) and size distribution (C) of FA-PCNP@DOX in PBS solutions at pH 7.4, pH 5.3 and in serum. Values expressed were means ± SD of 3 independent experiments.

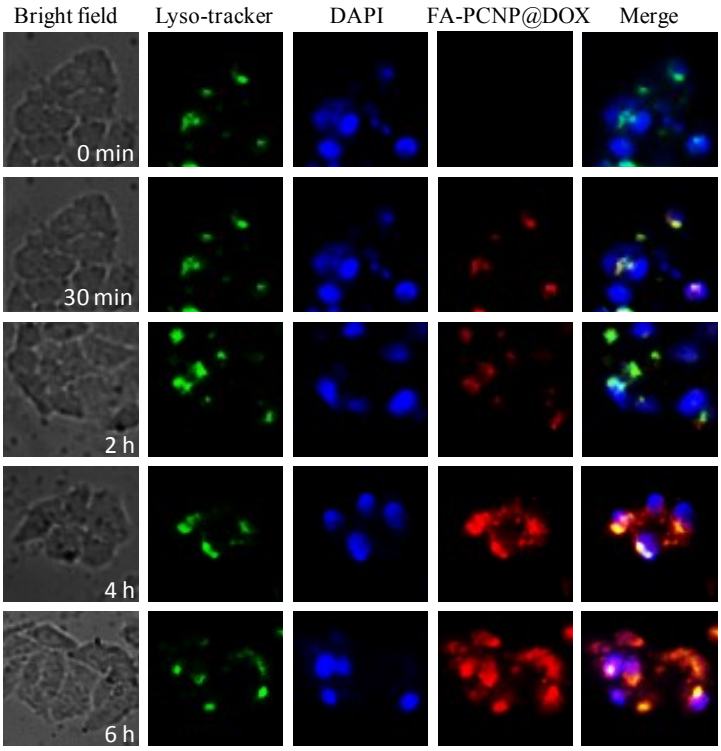

Figure S10. Intracellular trafficking of FA-PCNP@DOX in RHepG2 cells for 6 h, as detected by Lysotracker-DAPI staining.

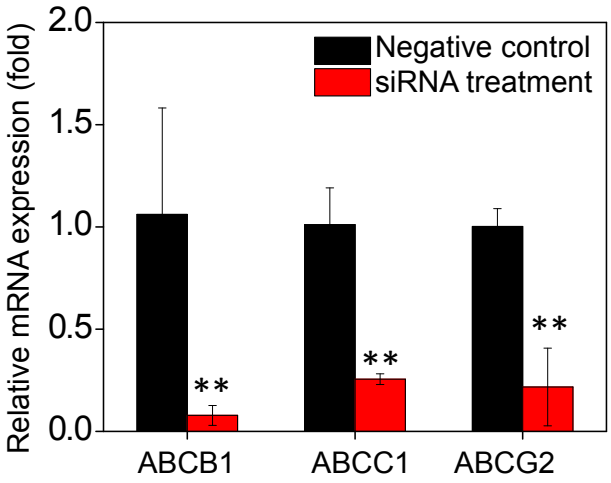

Figure S11. mRNA expression level of ABCB1, ABCC1 and ABCG2 in RHepG2 cells after treatment of siABCB1, siABCC1 and siABCG2. The RHepG2 cells were treated with 400 ng/mL of control siRNA, siABCB1, siABCC1 or siABCG2 respectively for 24 h in FBS-free DMEM. ** represented the statistical difference of mRNA expression between negative control and siRNA treatment group at $P < 0.01$ level.

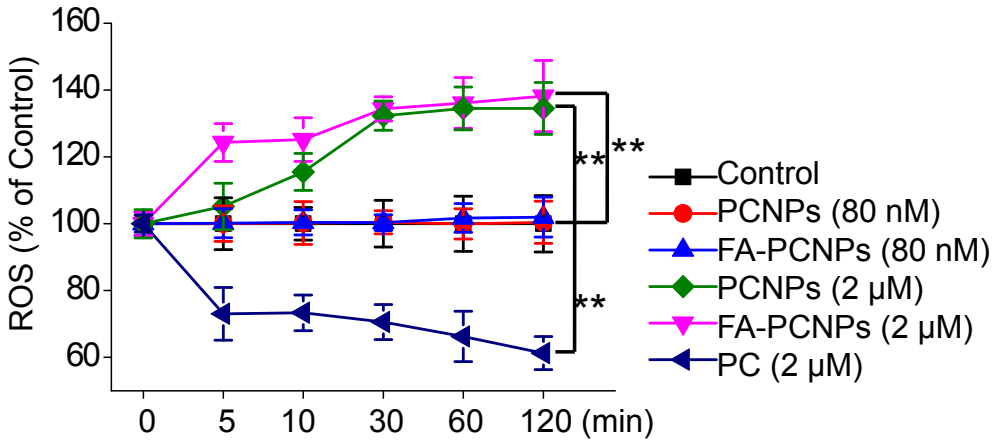

Figure S12. Intracellular ROS generation in RHepG2 cells after treatment of PC solution, PCNPs and FA-PCNPs with different concentrations. ** indicated the statistical difference between treatment group and control group at $P < 0.01$ level. Values expressed were means ± SD of 3 independent experiments.

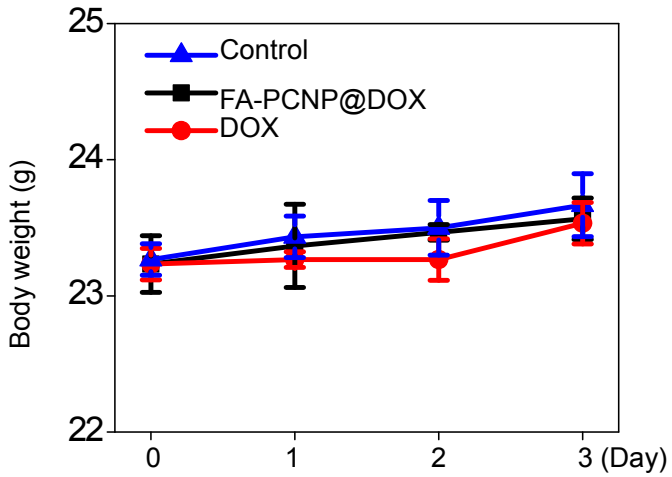

Figure S13. Body weight of nude mice after treated with 2.5 mg/kg FA-PCNP@DOX or DOX for 3 days of treatment, n=3.

Table S1. The ESI-MS analysis of compound (1) FA and (2) FA-EDC.

Complexes	Theoretical value (m/z)	Measured value (m/z)	Belonging to (m/z)
(1)	883.4	883.45	$[2M+H]^+$
	442.14	442.34	$[M+H]^+$
	328.11	327.61	$[M-C_5H_7O_4+NH_4]^+$
(2)	882.28	882.81	$[2M]^+$
	613.31	613.83	$[M+EDC+NH_4]^+$
	441.14	441.51	$[M]^+$

Table S2. *In vitro* release kinetic models of DOX from FA-PCNP@DOX.

	Model	Equation	R^2
Serum	Zero-order	Q = 1.142 + 0.0408t	0.7523
	First-order	ln(3.025-Q) =1.107-0.1310t	0.7877
	Higuchi	$Q =0.5170+0.3896t^{1/2}$	0.8925
	Ritger-peppas	lnQ =0.3122lnt-0.05881	**0.9311**
pH 7.4	Zero-order	Q = 1.846 + 0.0729t	0.7094
	First-order	ln(5.518-Q) = 1.708-0.1018t	0.9281
	Higuchi	$Q = 0.6474+0.7164t^{1/2}$	0.9014
	Ritger-peppas	lnQ =0.3612 +0.3483 lnt	**0.9383**
pH 5.3	Zero-order	Q = 32.46+ 0.8562t	0.6237
	First-order	ln(71.67-Q) = 4.272-0.1823t	0.9172
	Higuchi	$Q =17.26+8.696t^{1/2}$	0.8607
	Ritger-peppas	lnQ=3.358+0.2495lnt	**0.9787**

Table S3. Pharmaceutical parameters of FA-PCNP@DOX and DOX after iv injection at an equivalent dose of 4.21 mg DOX per kg of mouse body weight.

Parameter	FA-PCNP@DOX	DOX
$t_{½β}$ (h)	30.1	19.0
$AUC_{0\text{-}48\ h}$ (μg/L*h)	56847.8	4688.5
C_{max} (μg/L)	1356.7	688.9
Cl (mL/h)	5.1	60.7

$t_{½β}$, elimination phase, half-life period of medicine.

$AUC_{0\text{-}48\ h}$, area under curve.

C_{max}, maximum concentration observed.

Cl, clearance of medicine.