

Supplementary information

Nanoparticle delivery of chemotherapy combination regimen improves the therapeutic efficacy in mouse models of lung cancer.

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Supplemental Methods

Materials

Poly(lactic acid-*co*-glycolic acid)-poly(ethylene glycol) (36,000 Da-3000 Da, LA/GA =1/1, Cat. No. AK29) was purchased from PolySciTech, Inc. (West Lafayette, IN, USA). Dulbecco's phosphate buffer saline (DPBS) was purchased from Gibco by Life Technologies (Carlsbad, CA, USA). All other chemicals were purchased from Fisher Scientific or Sigma-Aldrich and used without further purification.

Preparation of CPPs.

CPPs were prepared as previously described.^{17,19,21} Briefly, CP was oxidized to the precursor Pt(IV) complex, $\text{Pt}(\text{NH}_3)_2(\text{Cl})_2(\text{OH})_2$, using a tenfold excess of hydrogen peroxide in water and then collected via crystallization. The $\text{Pt}(\text{NH}_3)_2(\text{Cl})_2(\text{OH})_2$ complex was then acetylated with a range of lipid anhydrides. Butyric, octanoic, and decanoic anhydrides produced octahedral CPPs modified at the axial positions with fatty acid chains ranging from C₄ (C₄CP), C₈ (C₈CP), and C₁₀ (C₁₀CP), respectively.

Characterization of PLGA-PEG NPs.

NPs' size (nm) and surface charge (ζ -potential, mV) were characterized using a Zetasizer Nano Z dynamic light scattering detector (Malvern Instruments Ltd, Worcestershire, UK). Prior to the measurements, NPs were diluted to 1 mg/mL with PBS. All reported values were based on the average of three separate measurements (Fig. S2).

Transmission electron microscopy (TEM) images were recorded using a JEOL 100CX II TEM (Fig. S3). Prior to TEM imaging, the concentrated NP samples were diluted by mixing with deionized water and negatively stained with 2% uranyl acetate on a

400-mesh carbon filmed copper grid. Excess NP dispersion was removed from the grid by dabbing with filter paper.

HPLC determination of drug concentration

NPs (10 μ L) were mixed with acetonitrile (50 μ L) and left overnight at 5 $^{\circ}$ C to destroy the NP structure. Drug amounts were measured using a Shimadzu SPD-M20A high-performance liquid chromatography (HPLC) instrument equipped with a diode array detector and a reverse-phase GP-C₁₈ column (Sepax Technology Inc., Newark, DE, USA) (Fig. 1, Fig. S2). Drugs were eluted using a binary solvent system (A:B, A- H₂O, B- CH₃CN, flow rate- 0.25 mL/min) that linearly increased from 0% to 100% B over 20 min (0-20 min), held at 100% B for an additional 5 min (20-25 min), and then reduced to 50% B for the final 5 min (25-30 min). Eluents were monitored at 230 nm for DTX and 245 nm for CPPs. Retention times were as follows: DTX- 11.6 min, C₄CP- 5.9 min, C₈CP- 12.9 min, and C₁₀CP- 15.9 min. Drug concentrations were determined using area under the curve integration in conjunction with DTX and CPP standard curves.

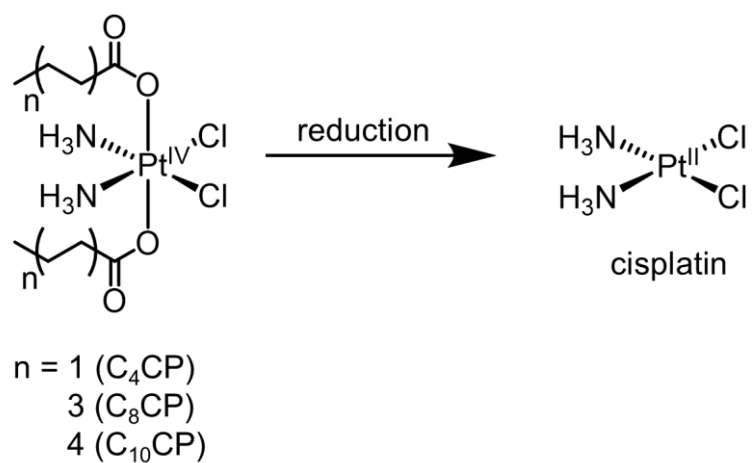


Figure S1. CPP structure and their conversion to form free cisplatin.

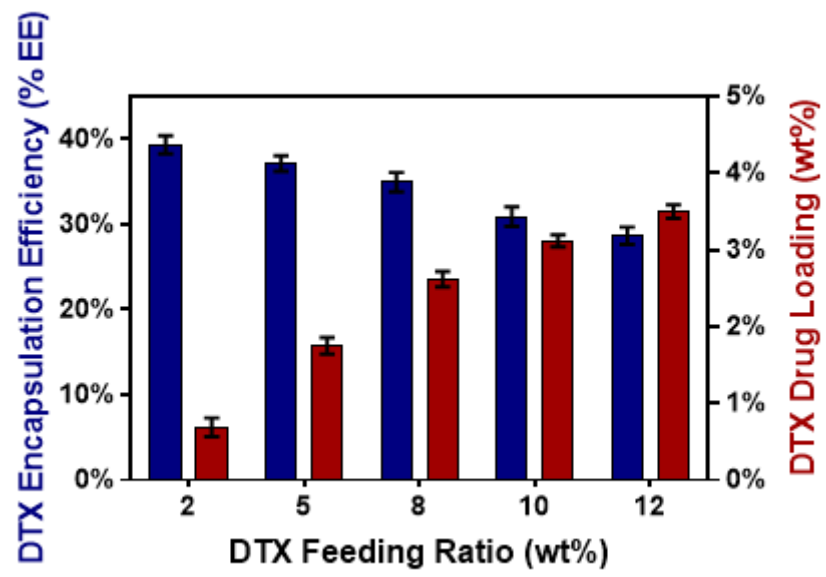


Figure S2. Encapsulation efficiency (%EE) and total drug loading (wt%) of singly loaded, DTX NPs.

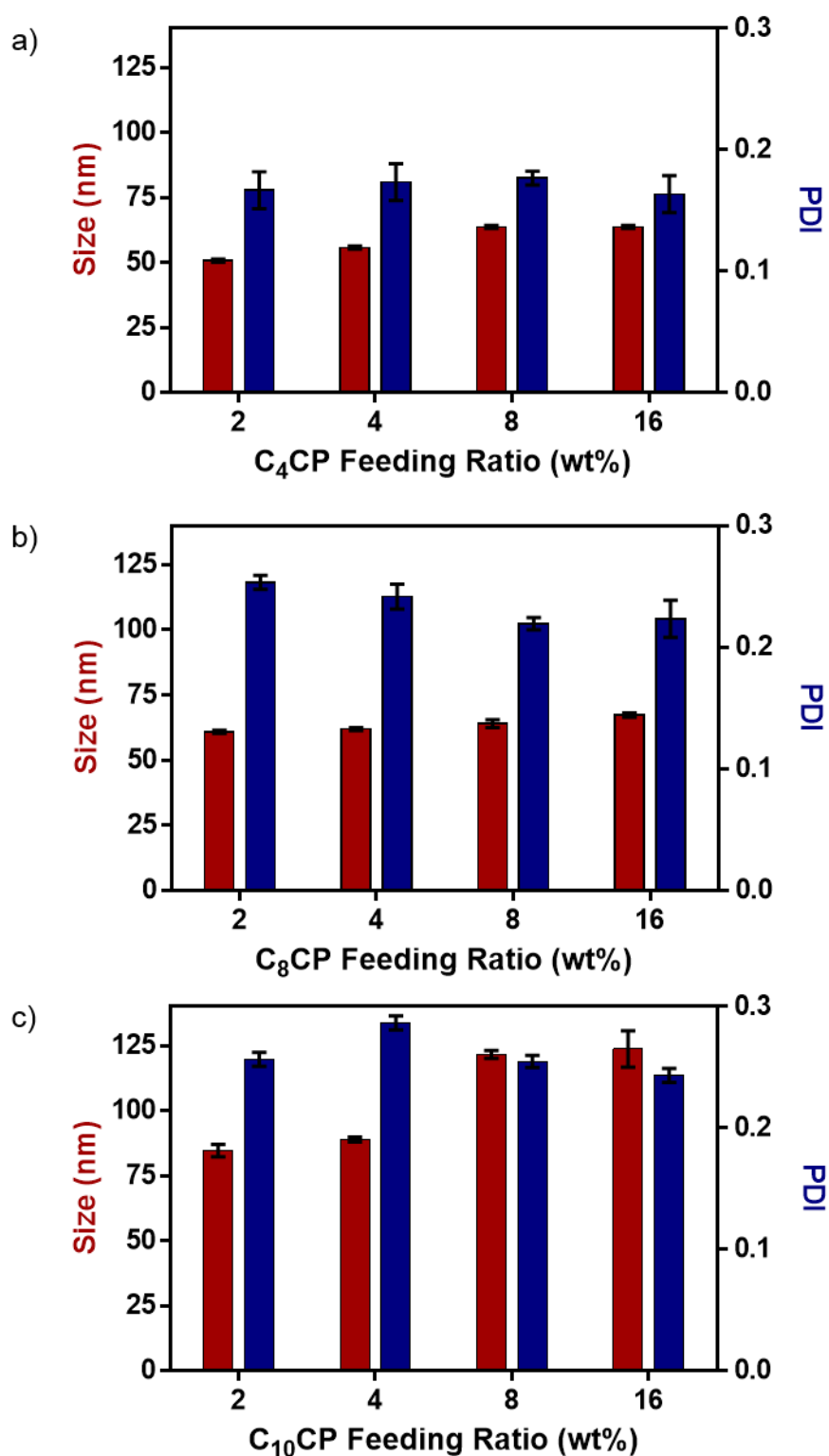


Figure S3. Size and PDI of dually loaded NPs containing DTX (10 %FR) and a) C₄CP, b) C₈CP, or c) C₁₀CP at a range of %FR.

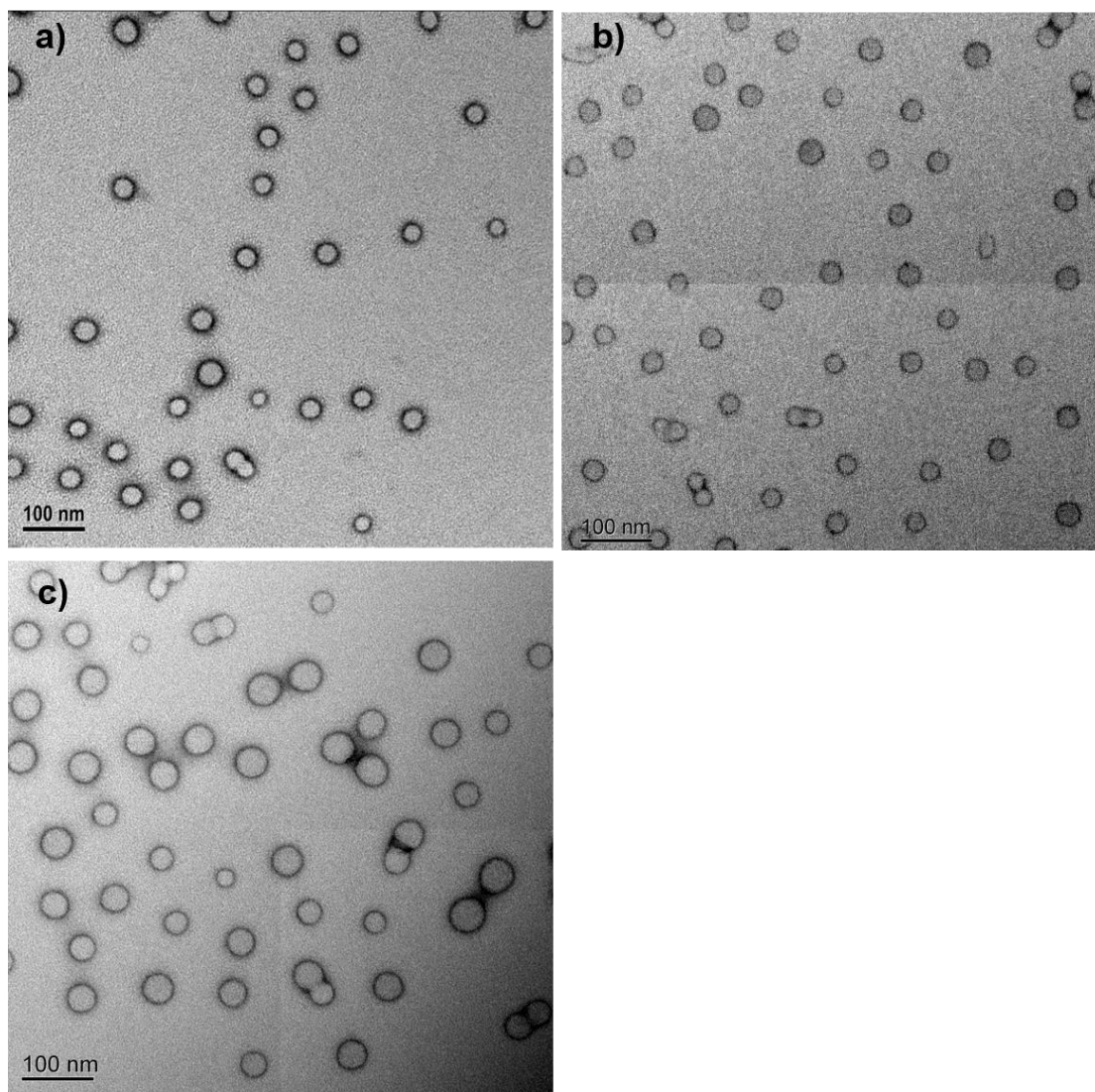


Figure S4. Representative TEM images of a) DTX:C₄CP NPs (1.2:1), b) DTX:C₈CP NPs (1.2:1), and c) DTX:C₁₀CP NPs (1.5:1).

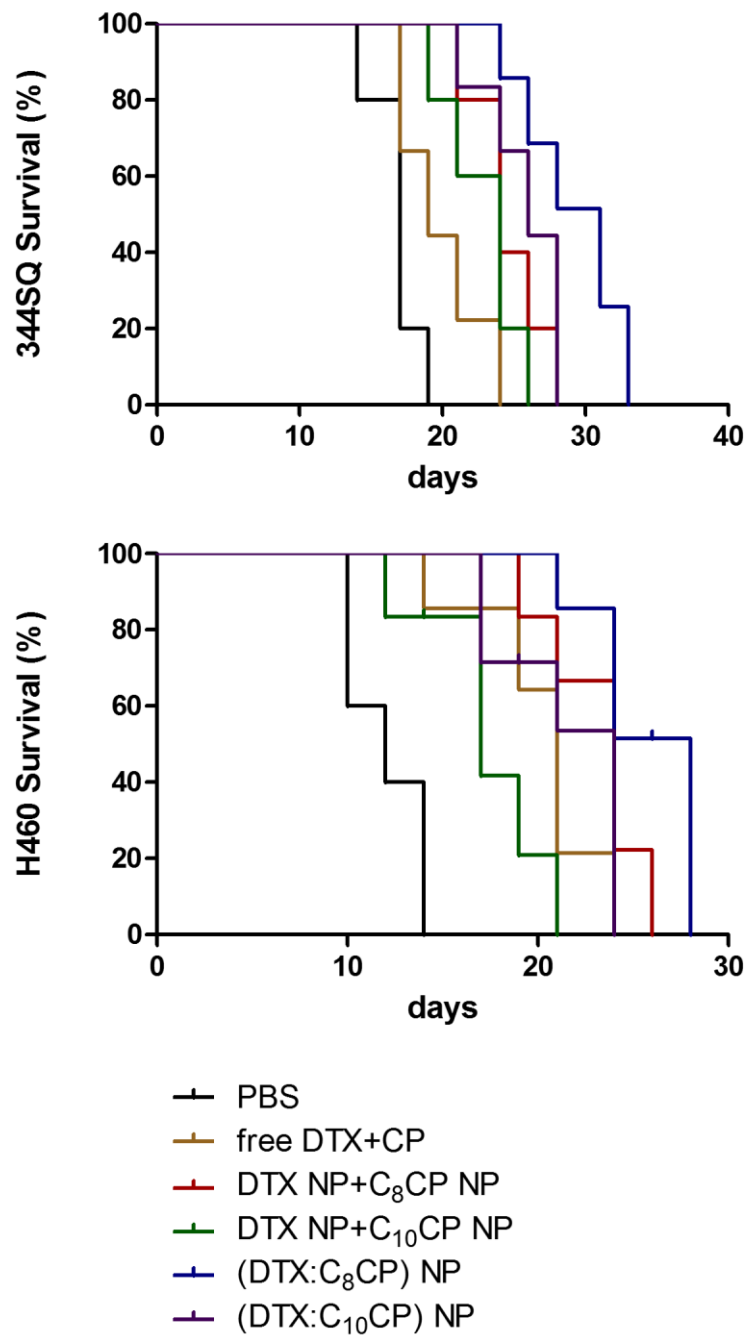


Figure S5. Survival curves for xenograft tumor models during treatment with combinations of free drugs and NPs.

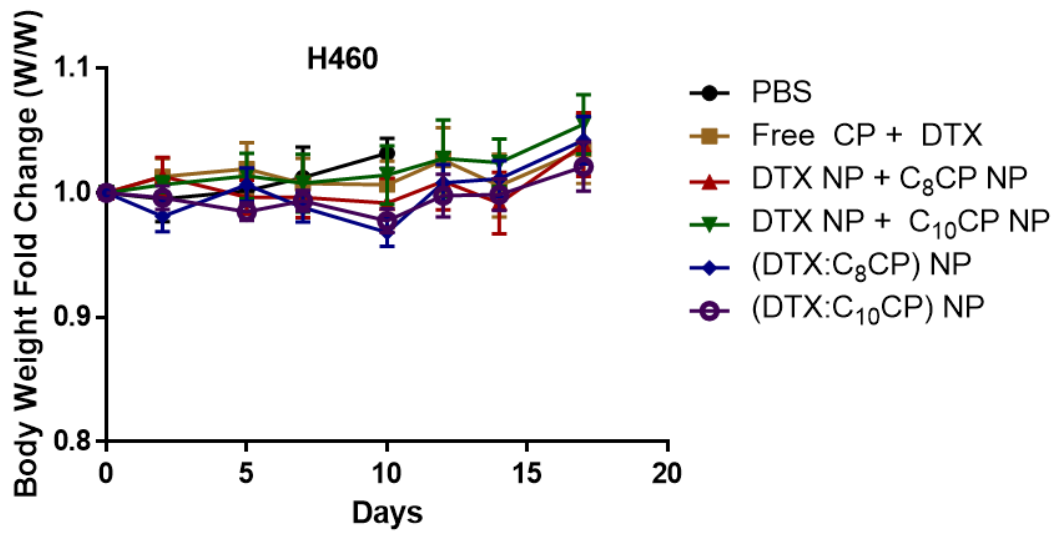
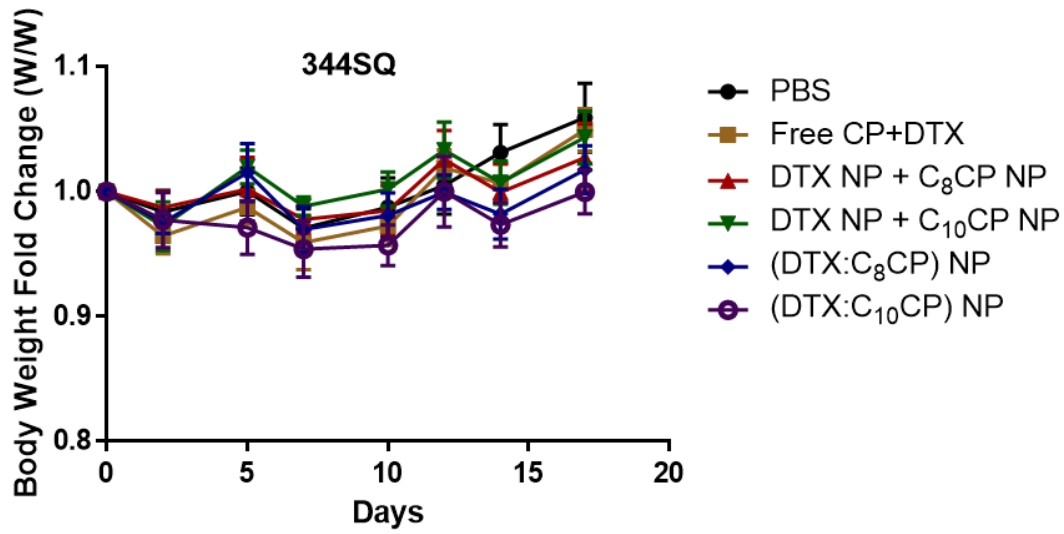


Figure S6. Murine body weight change during treatment.

Table S1. Loading characteristics of singly loaded NPs with CPPs or DTX.

Drug %FR	Drug loading (% wt) ^a			
	[%EE] ^b			
	C ₄ CP	C ₈ CP	C ₁₀ CP	DTX
2	0.23±0.08 [28.75±0.98]	0.65±0.19 [32.29±1.10]	0.56±0.21 [35.81±0.22]	0.69±0.12 [39.35±1.05]
5	0.66±0.17 [17.86±0.77]	1.14±0.21 [22.84±2.12]	1.66±0.22 [30.13±0.51]	1.75±0.11 [37.15±0.90]
8	1.15±0.09 [14.73±0.83]	1.72±0.12 [19.26±1.92]	2.59±0.12 [27.03±0.49]	2.62±0.10 [34.96±1.14]
10	1.09±0.13 [10.74±2.56]	1.93±0.10 [15.23±1.04]	2.98±0.11 [24.22±0.32]	3.12±0.08 [30.93±1.19]
12	1.12±0.14 [8.81±1.44]	1.68±0.05 [11.58±0.95]	3.39±0.19 [22.83±0.15]	3.50±0.09 [28.65±1.03]

a. Calculated as the percent weight of drug to polymer of nanoparticle. Determined by HPLC. b. Calculated as the % drug encapsulated versus the amount fed (%FR).

Table S2. CPP loading in dually loaded NPs at various %FR.^a

CPP %FR	Drug loading(% wt) ^b		
	[%EE] ^c		
	C ₄ CP	C ₈ CP	C ₁₀ CP
2	0.33±0.07	0.71±0.12	1.10±0.08
	[29.17±0.63]	[32.37±1.62]	[38.84±0.31]
4	0.76±0.22	1.05±0.10	1.80±0.16
	[20.46±2.78]	[29.23±0.94]	[36.78±0.62]
8	1.21±0.09	1.78±0.16	2.73±0.10
	[15.08±1.52]	[20.74±0.38]	[30.72±0.88]
16	1.28±0.13	1.69±0.13	3.48±0.15
	[8.72±0.77]	[11.94±1.25]	[20.00±0.35]

a. Loaded with a constant 10 %FR of DTX b. Calculated as the percent weight of drug to polymer of nanoparticle. Determined by HPLC. c. Calculated as the % drug encapsulated versus the amount fed (%FR).

Table S3. DTX^a loading in dually loaded NPs at varying %FR of CPPs.

CPP %FR	DTX drug loading(% wt) ^b		
	[%EE] ^c		
	C ₄ CP	C ₈ CP	C ₁₀ CP
2	3.11±0.18	3.14±0.21	3.15±0.15
	[31.19±0.99]	[32.31±1.98]	[32.34±0.62]
4	3.14±0.13	3.19±0.17	3.22±0.14
	[32.66±1.84]	[33.05±0.79]	[33.85±0.43]
8	3.17±0.16	3.28±0.18	3.27±0.14
	[33.57±0.85]	[34.66±1.35]	[34.56±0.81]
16	3.21±0.24	3.20±0.15	3.29±0.12
	[34.21±0.41]	[32.86±1.41]	[37.86±0.49]

a. Held at a constant 10 %FR DTX. b. Calculated as the percent weight of drug to polymer of nanoparticle. Determined by HPLC. c. Calculated as the % drug encapsulated versus the amount fed (%FR).

Table S4. Relative IC₅₀ value of each drug formulation

Formulation	Total Drug (nM)		Cisplatin (nM)		Docetaxel (nM)	
	H460	344SQ	H460	344SQ	H460	344SQ
Free CP	5,800	12,270	580	2270	-	-
Free DTX	80	190	-	-	80	190
Free DTX + CP (1.5:1)	52	160	21	65	31	95
Free DTX + CP (1.2:1)	60	152	27	69	33	83
DTX NP	95	220	-	-	95	220
C ₈ CP NP	188	680	188	680	-	-
DTX NP + C ₈ CP NP (1.2:1)	26	88	12	40	14	48
(DTX:C ₈ CP) NP (2.2:1)	37	100	12	31	34	163
(DTX:C ₈ CP) NP (1.2:1)	25	90	11	41	14	49
(DTX:C ₈ CP) NP (.8:1)	44	158	24	88	20	70
C ₁₀ CP NP	78	150	78	150	-	-
DTX NP + C ₁₀ CP NP (1.5:1)	19	73	8	30	11	43
(DTX:C ₁₀ CP) NP (1.5:1)	18	70	7	28	11	42
(DTX:C ₁₀ CP) NP (1:1)	22	100	11	51	11	49
(DTX:C ₁₀ CP) NP (.8:1)	27	130	15	71	12	56

Table S5. Hematologic toxicity of small-molecule and NP encapsulated drugs.^a

Cell Line	Treatment Arm	White blood cells ($\times 10^3/\mu\text{l}$)	Red blood cells ($\times 10^6/\mu\text{l}$)
Normal Range	-	2.6-10.1	6.5-10.1
344SQ	PBS	6.6	8.46
	Free DTX + CP	2.6	6.53
	DTX NP + C ₈ CP NP (1.2:1)	4.1	8.32
	DTX NP + C ₁₀ CP NP (1.5:1)	2.9	7.09
	(DTX:C ₈ CP)NP (1.2:1)	5.4	7.70
	(DTX:C ₁₀ CP)NP (1.5:1)	3.5	6.88
H460	PBS	5.7	9.02
	Free DTX + CP	2.8	6.66
	DTX NP + C ₈ CP NP (1.2:1)	4.2	8.21
	DTX NP + C ₁₀ CP NP (1.5:1)	3.3	7.37
	(DTX:C ₈ CP)NP (1.2:1)	4.8	7.87
	(DTX:C ₁₀ CP)NP (1.5:1)	3.2	7.54

^aHematologic toxicity parameter was determined for one mouse in each group 4 days after the last I.V. injection.

Table S6. Hepato and renal toxicity of small-molecule and encapsulated drugs.^a

Cell Line	Treatment Arm	Plasma AST (Units/L)	Plasma ALT (Units/L)	BUN (mg/dL)	Crea (mg/dL)
Normal Range ^b	-	54-298	17-132	12-33	0.2-0.9
344SQ	PBS	62	41	23	0.2
	Free DTX + CP	84	45	26	0.3
	DTX NP + C ₈ CP NP (1.2:1)	63	39	24	0.2
	DTX NP + C ₁₀ CP NP (1.5:1)	62	41	25	0.2
	(DTX:C ₈ CP)NP (1.2:1)	57	38	23	0.2
	(DTX:C ₁₀ CP)NP (1.5:1)	64	43	24	0.2
H460	PBS	70	32	20	0.2
	Free DTX + CP	98	40	26	0.2
	DTX NP + C ₈ CP NP (1.2:1)	69	31	25	0.2
	DTX NP + C ₁₀ CP NP (1.5:1)	70	42	25	0.2
	(DTX:C ₈ CP)NP (1.2:1)	59	39	24	0.2
	(DTX:C ₁₀ CP)NP (1.5:1)	60	40	22	0.2

^aToxicity parameter was determined for one mouse in each group 4 days after the last I.V. injection. ^bFrom Ref. 1.

References

1. Miao L, Guo S, Zhang J, Kim WY, Huang L. Nanoparticles with Precise Ratiometric Co-Loading and Co-Delivery of Gemcitabine Monophosphate and Cisplatin for Treatment of Bladder Cancer. *Adv Funct Mater.* 2014, 24(42): 6601-6611.