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


# Tumor pH-Triggered "Charge Conversion" Nanocarriers with On-Demand Drug Release for Precise Cancer Therapy

Bo-Ai Ma[†, ‡], Chun-Yang Sun [†,*]

[†] Department of Radiology and Tianjin Key Laboratory of Functional Imaging, Tianjin Medical University General Hospital, Tianjin 300052, P.R. China

[‡] School of Food and Biological Engineering, Hefei University of Technology, Hefei, Anhui 230009, P.R. China

E-mail: chysunshine@gmail.com (C. Y. Sun)

## Materials and Methods

### 1. Materials and characterization

(2-nitro-1H-imidazol-1-yl) methanethiol (NI-SH) and cyclic phosphate monomer allyl ethylene phosphate (AEP) were synthesized by a similar method described previously, and AEP was distilled under vacuum twice just before use.[1, 2] Cysteamine hydrochloride, succinic anhydride and 2,3- dimethylmaleic anhydride were purchased from Alfa Aesar (Shanghai, China). Tetrahydrofuran (THF) was refluxed over potassium-sodium alloy under $N_2$ atmosphere and distilled just before use. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and 1.5.7-triazabicyclo-[4.4.0]dec-5-ene (TBD) purchased from Sigma-Aldrich Chemical Co., Ltd.. Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from Life Technologies Corporation (Gibco, USA). All other reagents and solvents without statement were of analytical grade and used as received.

The proton nuclear magnetic resonance ($^1$H NMR) spectra were recorded in deuterated chloroform ($CDCl_3$) or deuterated dimethyl sulfoxide (DMSO-$d_6$) on a 400 MHz spectrometer (Avance III, Bruker, Germany). The size and zeta potential measurements were carried out in aqueous solution using a Malvern ZS90 dynamic light scattering instrument with a He-Ne laser (633 nm) and 90° collecting optics. The data were analyzed using Malvern Dispersion Technology Software 5.10.

### 2. Cells Lines and Animals

The human breast adenocarcinoma (MDA-MB-231) cells were obtained from the American Type Culture Collection (ATCC, MD, USA). The cells were cultured in

DMEM medium supplemented with 10% fetal bovine serum at 37 °C using a humidified 5% $CO_2$ incubator. The xenograft tumor model was generated by injection of $2\times10^6$ MDA-MB-231 cells (100 μL) with 20% Matrigel® Matrix (Corning, Bedford, MA) into the mammary fat pat of female BALB/c nude mice.

**3. Statistical analysis**

The statistical significance of treatment outcomes was assessed using Student's *t*-test (two-tailed); $p < 0.05$ was considered statistically significant in all analyses (95% confidence level).

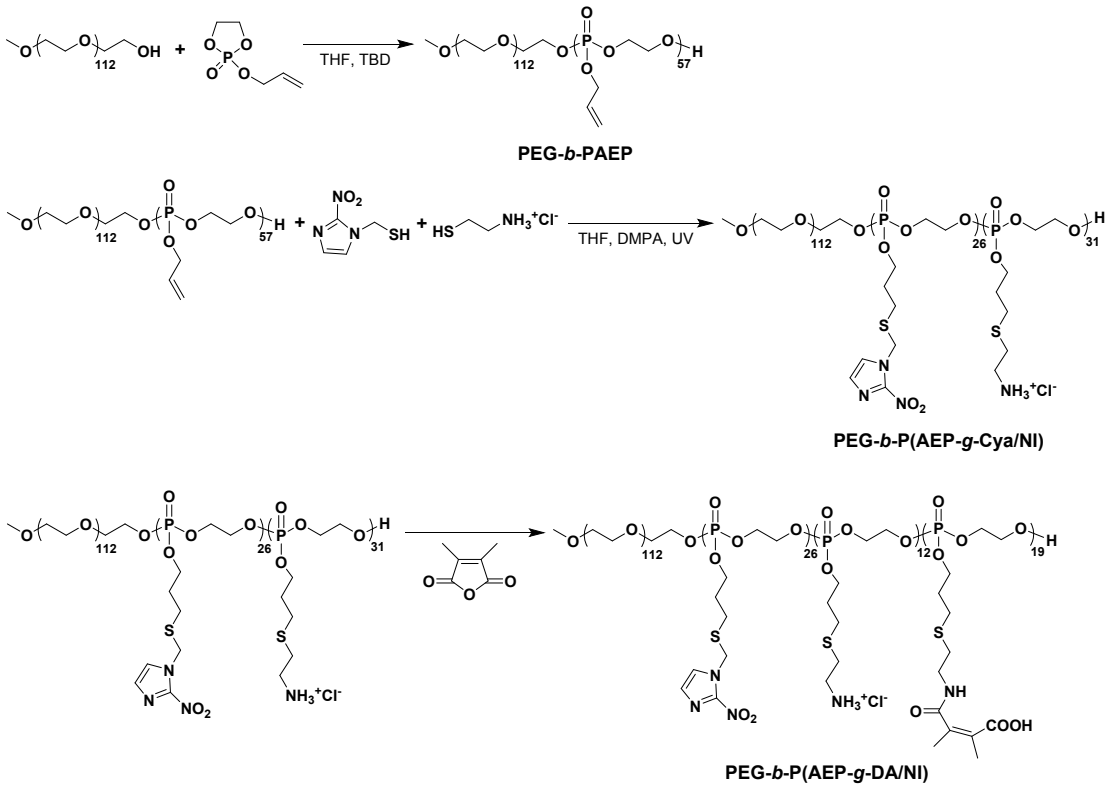


**Figure S1.** Synthetic route of PEG-*b*-P(AEP-*g*-DA/NI).

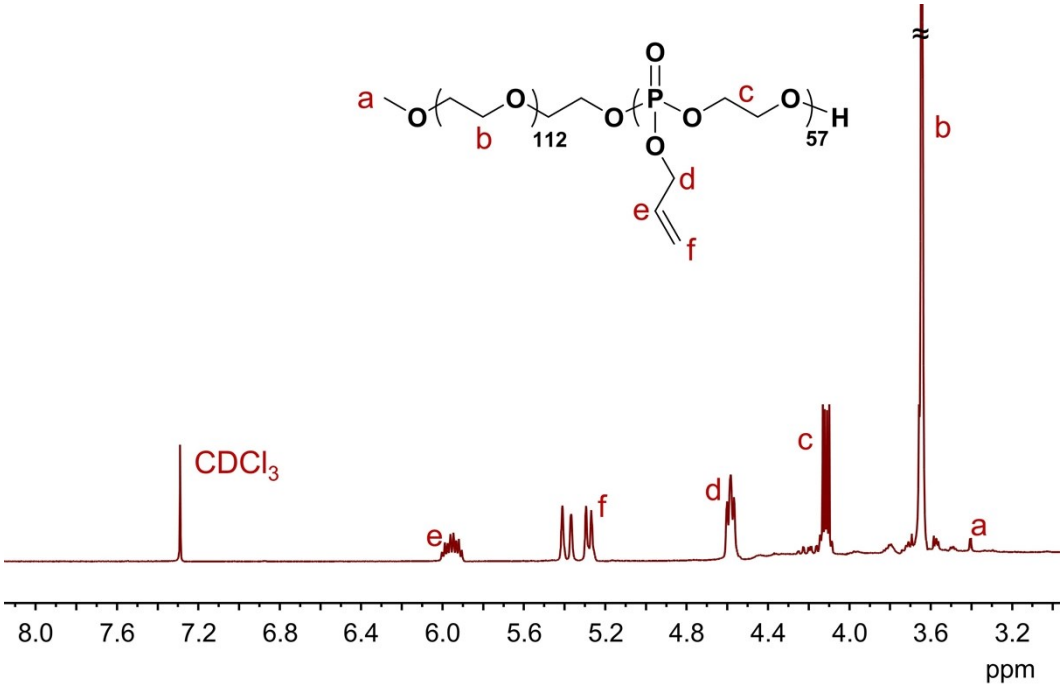


**Figure S2.** $^{1}$H NMR spectrum of PEG-*b*-PAEP in $CDCl_3$ recorded on an AVANCE III 400 MHz spectrometer at 25 °C.

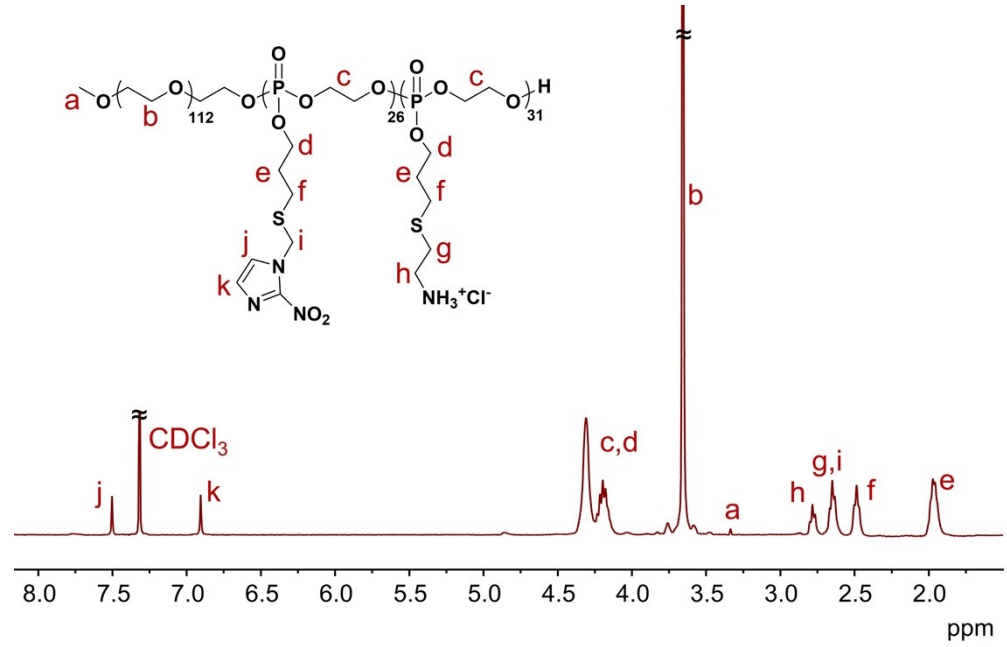


**Figure S3.** $^{1}$H NMR spectrum of PEG-*b*-P(AEP-*g*-Cya/NI) in $CDCl_3$ recorded on an AVANCE III 400 MHz spectrometer at 25 °C.

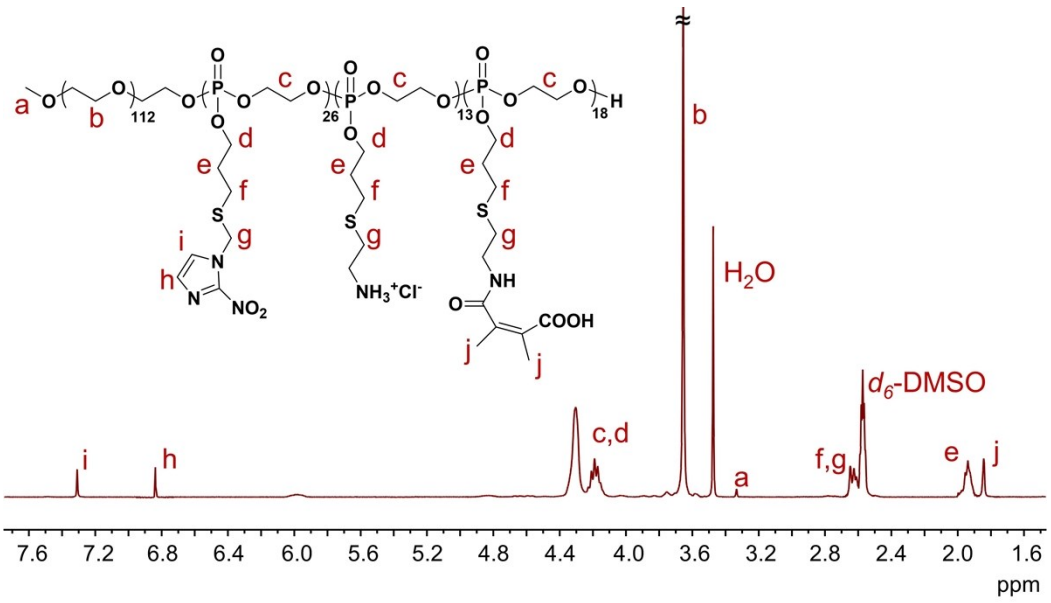


**Figure S4.** $^{1}$H NMR spectrum of PEG-*b*-P(AEP-*g*-DA/NI) in $d_6$-DMSO recorded on an AVANCE III 400 MHz spectrometer at 25 °C.

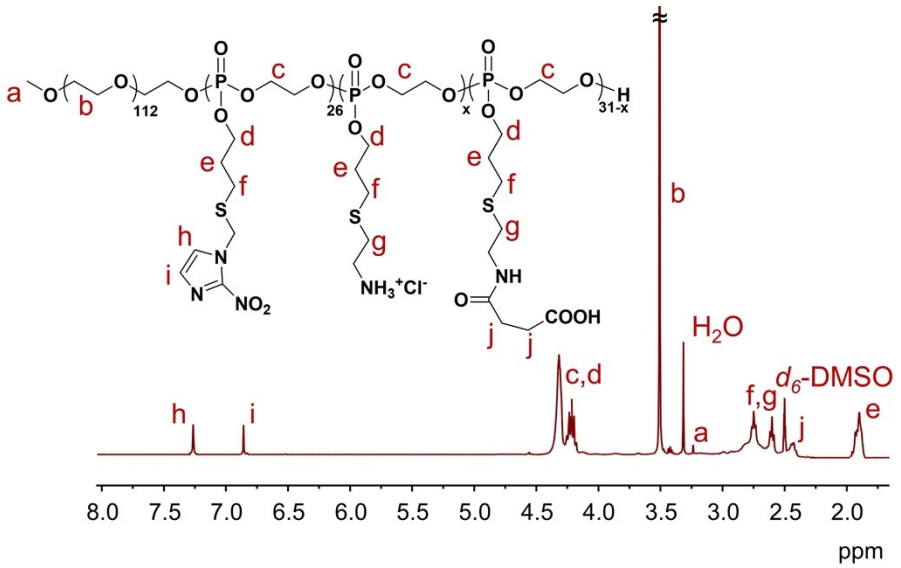


**Figure S5.** $^1$H NMR spectrum of PEG-*b*-P(AEP-*g*-SA/NI) in $d_6$-DMSO recorded on an AVANCE III 400 MHz spectrometer at 25 °C.

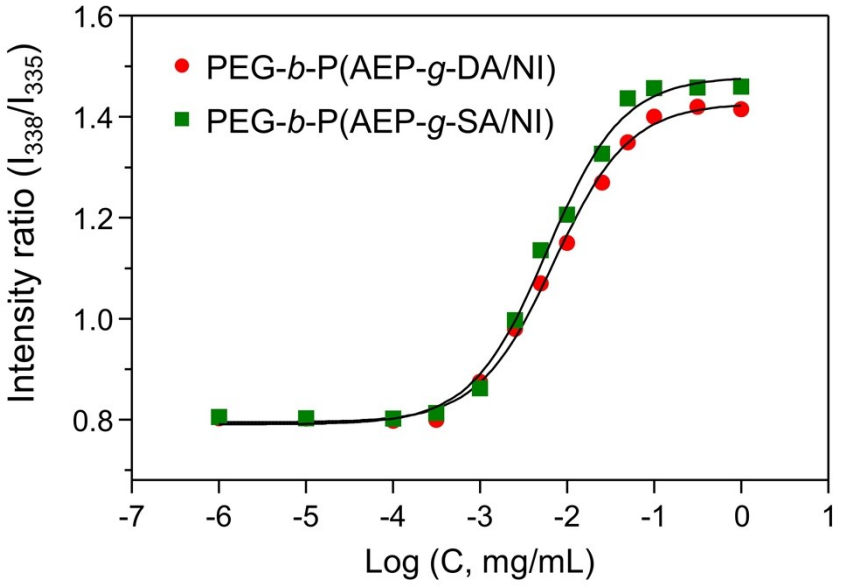


**Figure S6.** Plot of the $I_{338}/I_{335}$ ratio (pyrene fluorescence) against log C of polymeric micelles.

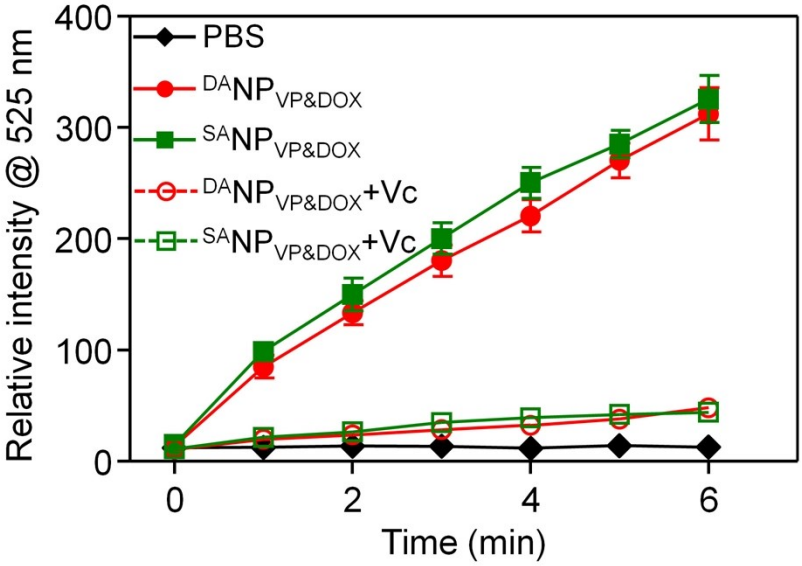


**Figure S7.** Relative fluorescence of SOSG at 525 nm in different samples with X-ray radiation.

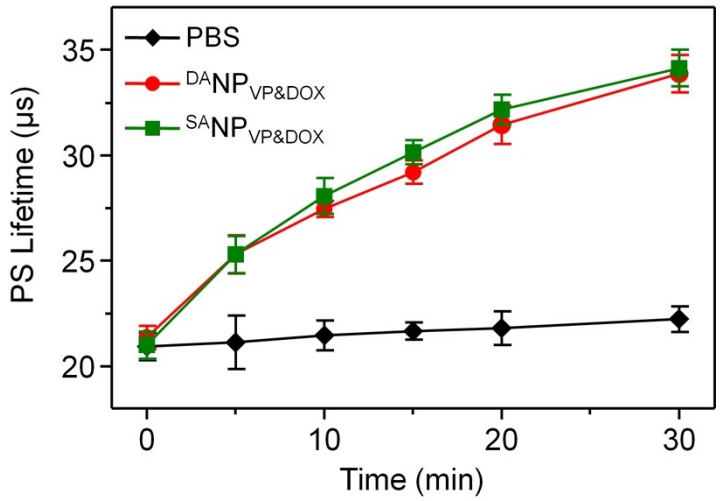


**Figure S8.** Phosphorescence lifetime *vs* irradiation time in PBS, $^{DA}NP_{VP\&DOX}$ or $^{SA}NP_{VP\&DOX}$.

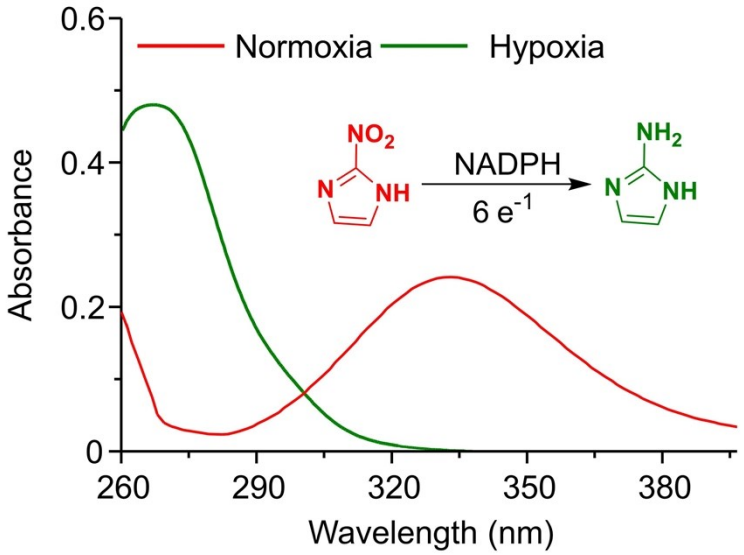


**Figure S9.** UV−vis spectra of $^{DA}$NP in HEPES buffer containing 100 μM NADPH under hypoxic (filled with $N_2$) or normoxic conditions for 24 h.

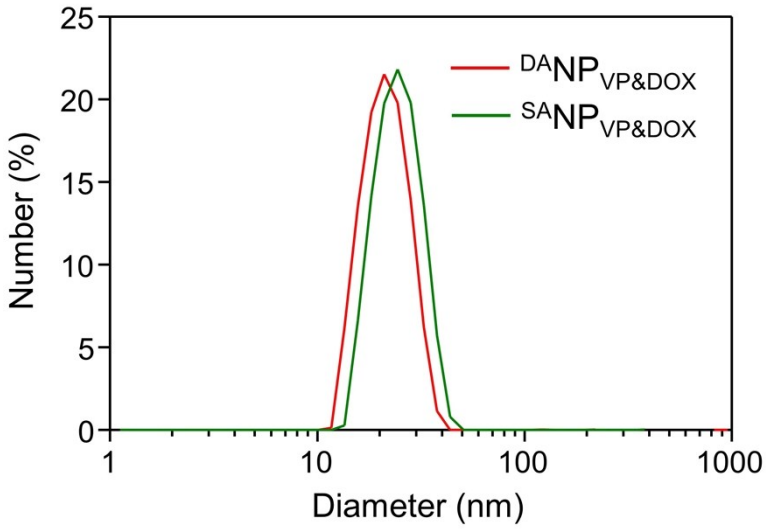


**Figure S10.** DLS measurement of $^{DA}NP_{VP\&DOX}$ or $^{SA}NP_{VP\&DOX}$ after X-ray radiation.

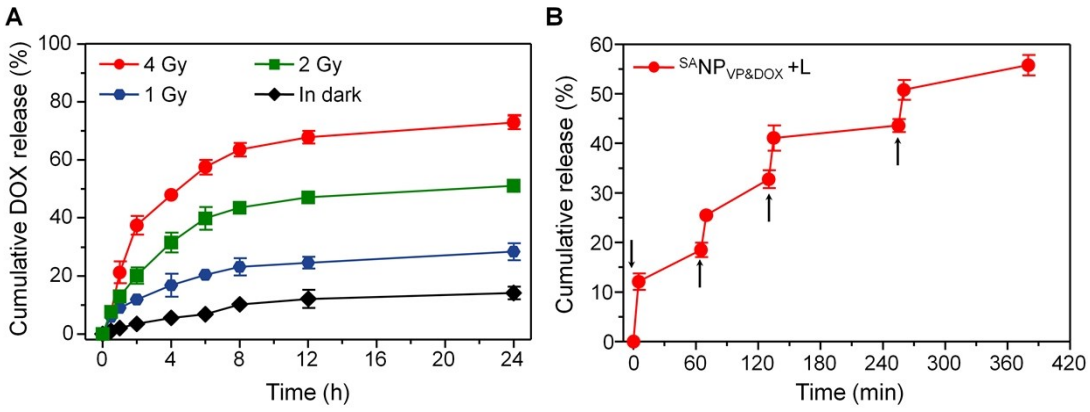


**Figure S11.** (A) DOX release profiles from $^{SA}NP_{VP\&DOX}$ following X-ray exposure at different doses. (B) X-ray-stimulated pulsed release of DOX from $^{SA}NP_{VP\&DOX}$. The samples were exposed with X-Ray for 5 min at different time points indicated by the arrows.

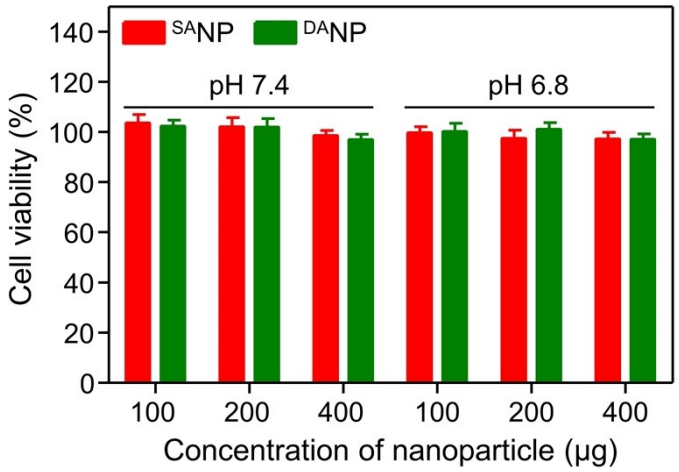


**Figure S12.** Relative MDA-MB-231 cell viabilities after incubation with $^{SA}NP$ or $^{DA}NP$ for 48 h.

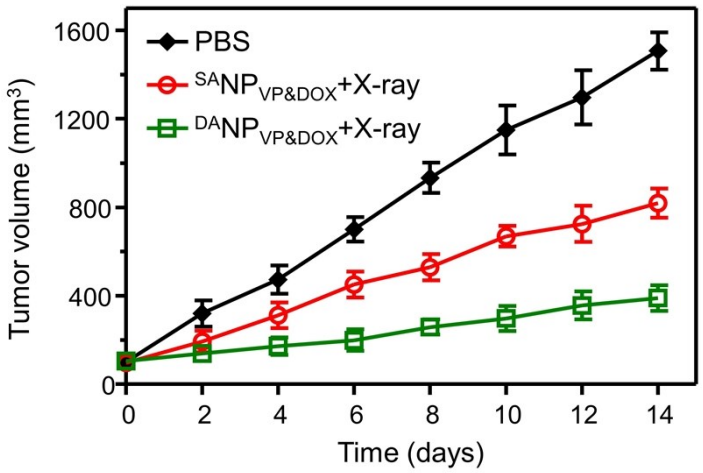


**Figure S13.** Tumor growth on mice bearing MDA-MB-231 tumor xenograft with different treatments (n = 5). The mice were received the irradiation of 4.0 Gy X-ray with the pork tissues of 1.5 cm thickness lain on top.

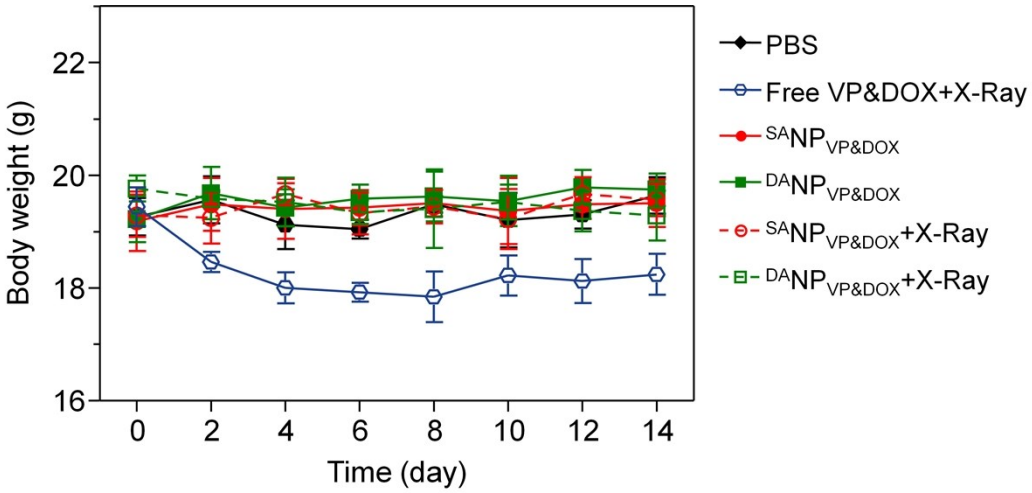


**Figure S14.** Body weight change of the mice during the therapeutic window.

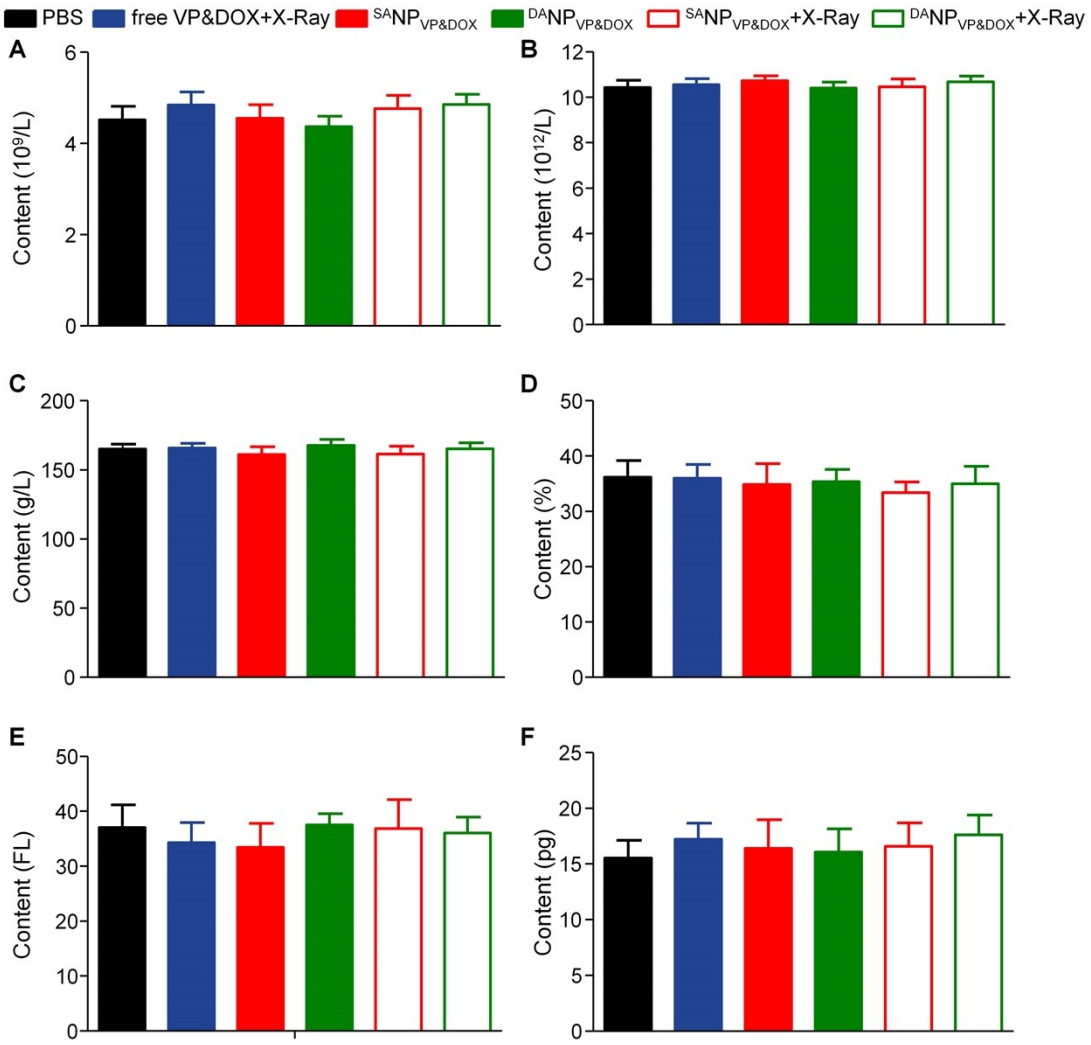


**Figure S15.** Hematology analysis of the mice after different treatments: (A) white blood cell (WBC), (B) red blood cell (RBC), (C) hemoglobin (HGB), (D) hematocrit (HCT), (E) mean corpuscular volume (MCV), and (F) mean corpuscular hemoglobin (MCH), respectively.

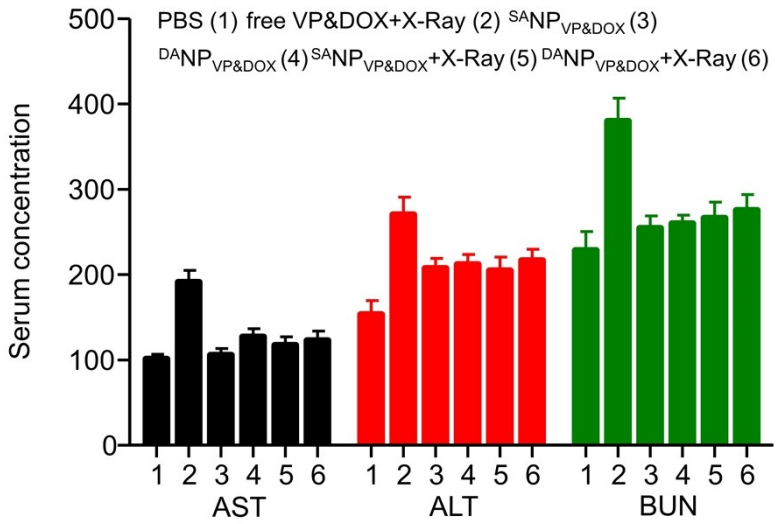


**Figure S16.** Enzyme-linked immunosorbent examination of mouse alanine aminotransferase (ALT, U/L), aspartate transaminase (AST, U/L) and blood urea nitrogen (BUN, 10 μmol/L) in the serum after receiving different treatments.

**Table S1.** Diameters, drug loading contents (DLCs) of VP and DOX for $^{SA}NP_{VP\&DOX}$ and $^{DA}NP_{VP\&DOX}$.

| Parameter | Diameter (nm) | | DLC (%) | |
|---|---|---|---|---|
| | DLS | TEM[a] | VP | DOX |
| $^{SA}NP_{VP\&DOX}$ | 128.8 | 121.15±11.91 | 4.34 | 3.96 |
| $^{DA}NP_{VP\&DOX}$ | 123.1 | 113.72±10.79 | 4.29 | 3.71 |

[a] Calculated by Image J software.

**References**

1. C. Qian, P. Feng, J. Yu, Y. Chen, Q. Hu, W. Sun, X. Xiao, X. Hu, A. Bellotti, Q.-D. Shen and Z. Gu, *Angewandte Chemie International Edition*, 2017, **56**, 2588-2593.

2. J.-Z. Du, X.-J. Du, C.-Q. Mao and J. Wang, *Journal of the American Chemical Society*, 2011, **133**, 17560-17563.