

SUPPORTING INFORMATION

Precision Synchronization of Hyperthermia-Chemotherapy: Photothermal induced On-Demand Release from Injectable Temperature Sensitive Hydrogels of Doxorubicin-Loaded Gold Nanocages

Jiangshan Wan,^{a,b†} Shinan Geng,^{a†} Hao Zhao,^{a†} Xiaole Peng,^a Jiabao Xu,^a Meihe Wei,^a Junxiong Mao,^a Yang Zhou,^a Quan Zhu,^b Yanbing Zhao ^{*a} and Xiangliang Yang ^{*a}

^a National Engineering Research Center for Nanomedicine, College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan 430074, P. R. China

^b Institute of Consun Co. for Chinese Medicine in Kidney Diseases, Consun Pharmaceutical Group, Guangzhou 510530, P. R. China

† J.S. Wan, S.N. Geng and H. Zhao contributed equally to this work and should be considered as co-first authors.

*Corresponding authors: Prof. Dr. Yanbing Zhao, E-mail: zhaoyb@hust.edu.cn; Prof. Dr. Xiangliang Yang, E-mail: yangxl@hust.edu.cn.

Supplementary Data

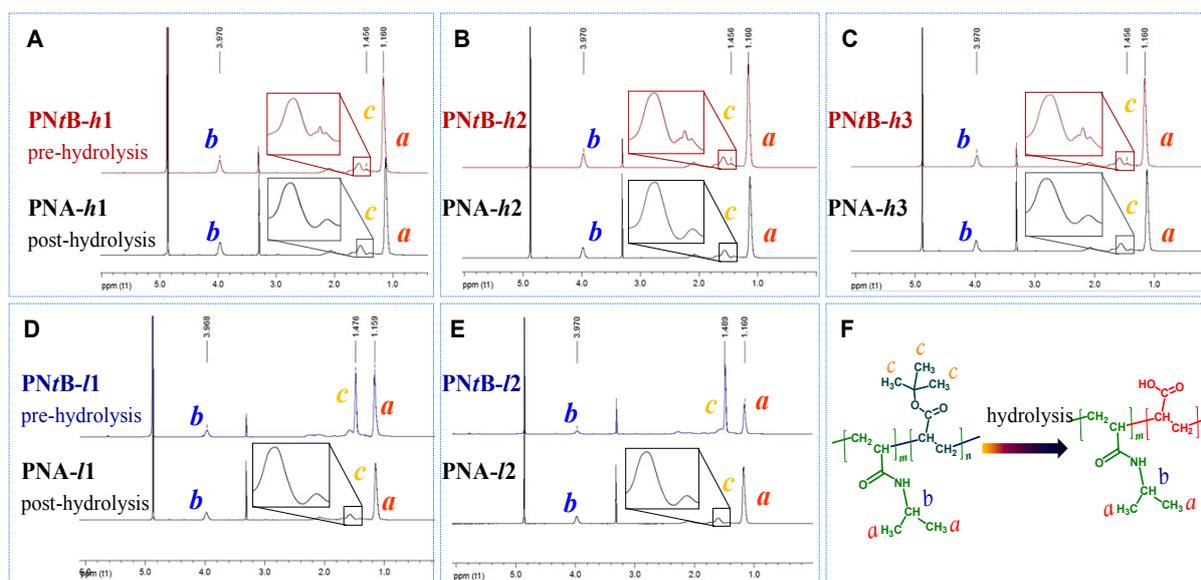


Fig. S1. ¹H-NMR spectra of various PNtB polymers before and after hydrolyzation: (A) PNtB-h1 (red) and PNA-h1 (black); (B) PNtB-h2 (red) and PNA-h2 (black); (C) PNtB-h3 (red) and PNA-h3 (black); (D) PNtB-l1 (red) and PNA-l1 (black); (E) PNtB-l2 (red) and PNA-l2 (black); (F) The hydrolyzation reaction of PNtBs to PNA polymers.

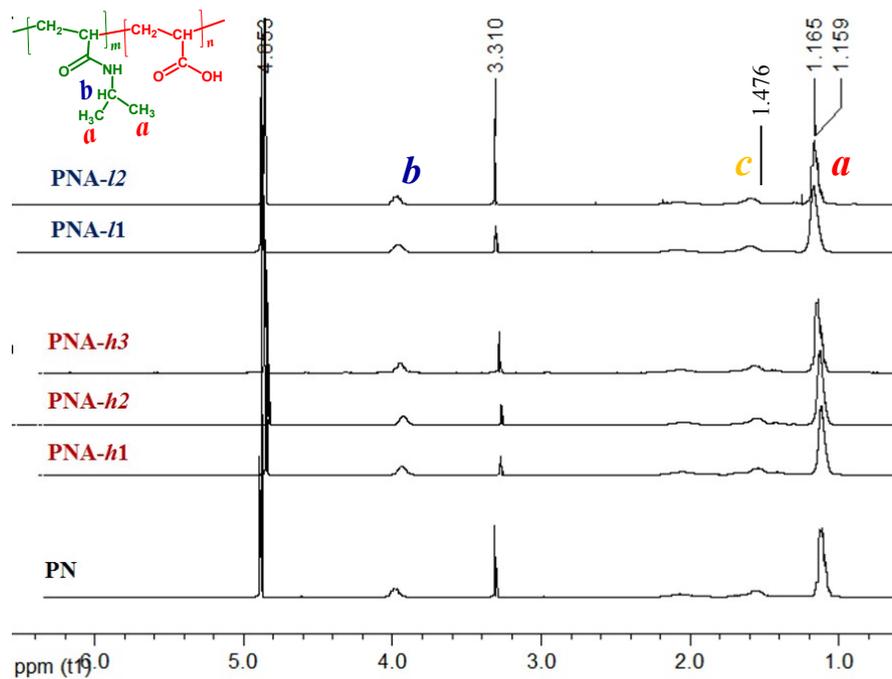


Fig. S2. $^1\text{H-NMR}$ spectrum comparison of PN polymers, three kind of PNA-*hs* and two kind of PNA-*ls*.

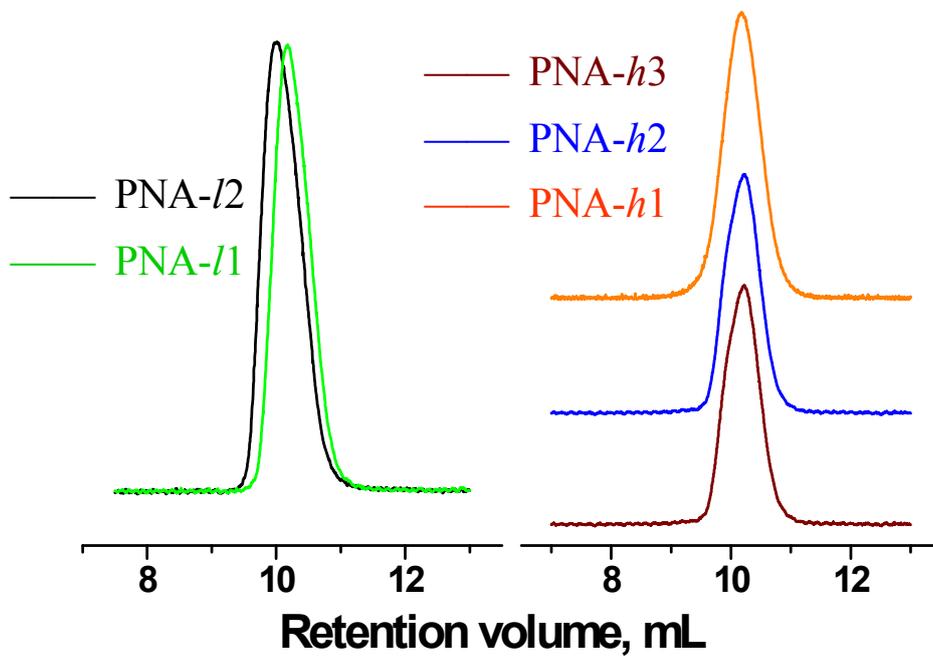


Fig. S3. Molecular weight characterization of two kind of PNA-ls (left plot) and three kind of PNA-hs (right plot) by gel penetration chromatography (GPC). Sampling concentration was 5.0 mg/mL. Polystyrene and THF were used as standards and eluent. Flowing rate: 1.0 mL/min; column temperature: 35 °C

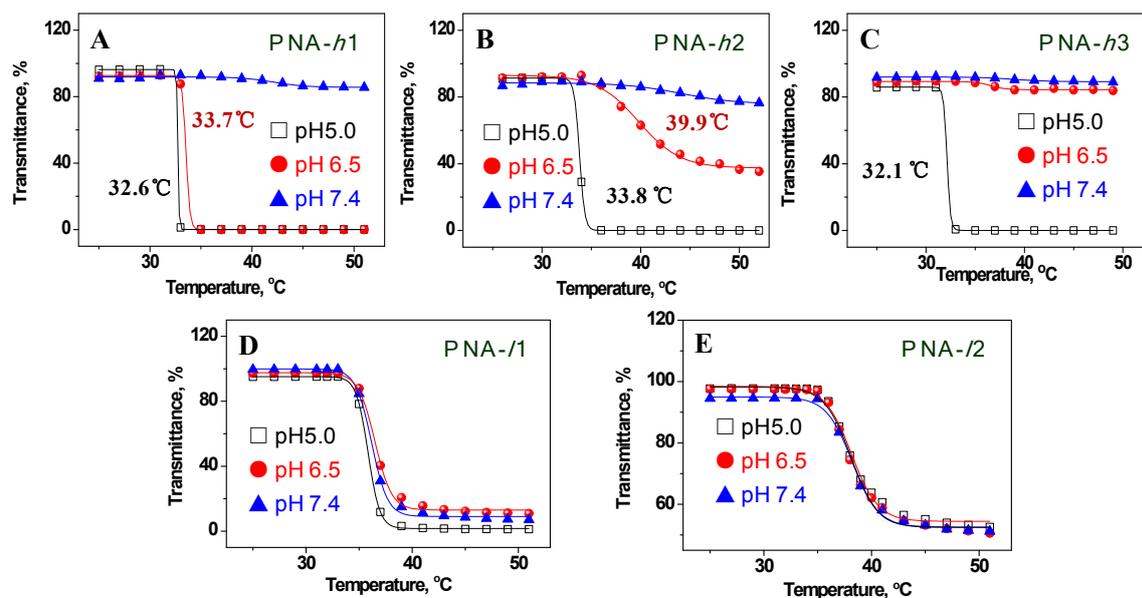


Fig. S4. Temperature-dependent transmittance of PNA-*hs* and PNA-*ls* using UV/Vis measurement at the wavelength of 500 nm at various pHs. (A) PNA-*h1*; (B) PNA-*h2*; (C) PNA-*h3*; (D) PNA-*l1*; (E) PNA-*l2*.

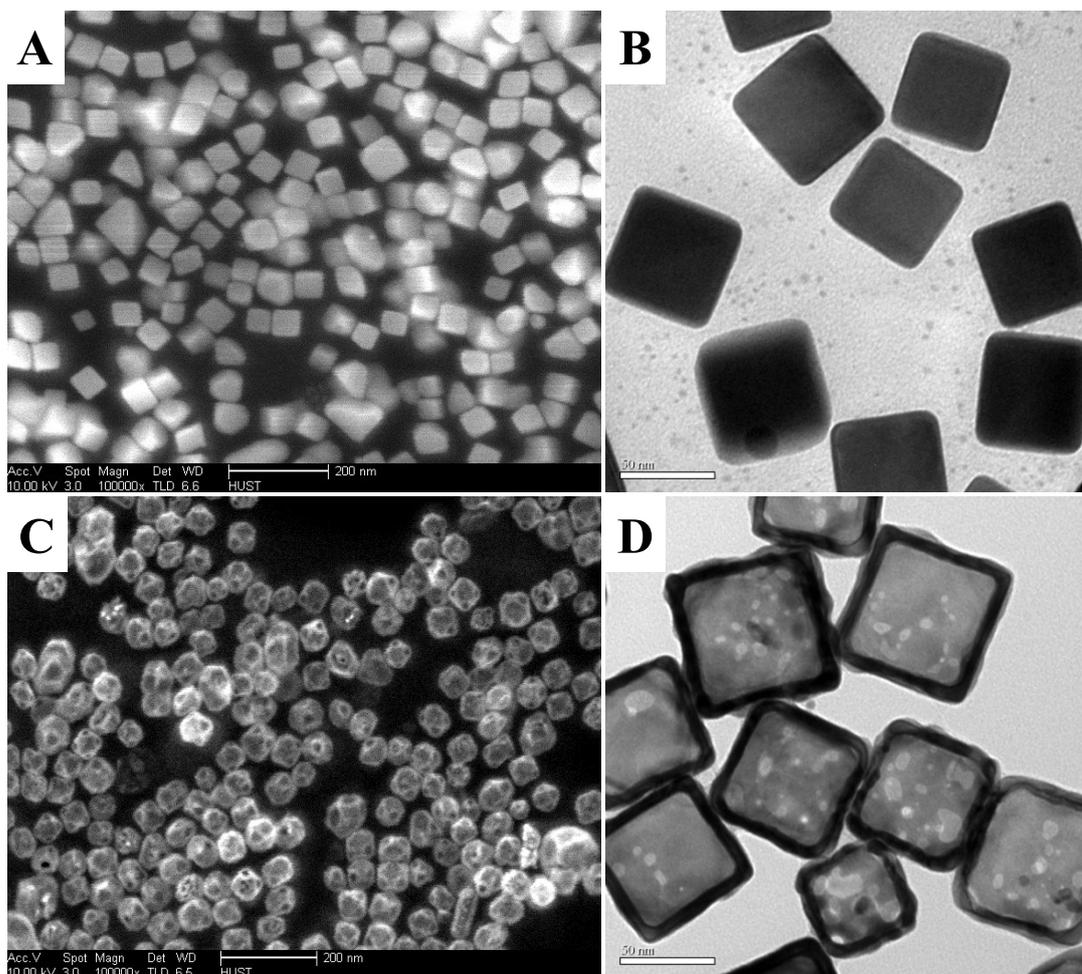


Fig. S5. Microstructure characterization: (A) and (B) were SEM and TEM photos of Ag nanocubes respectively; (C) and (D) were SEM and TEM photos of gold nanocages (GNCs) respectively. The bars of SEM and TEM were 200 nm and 50 nm, respectively.

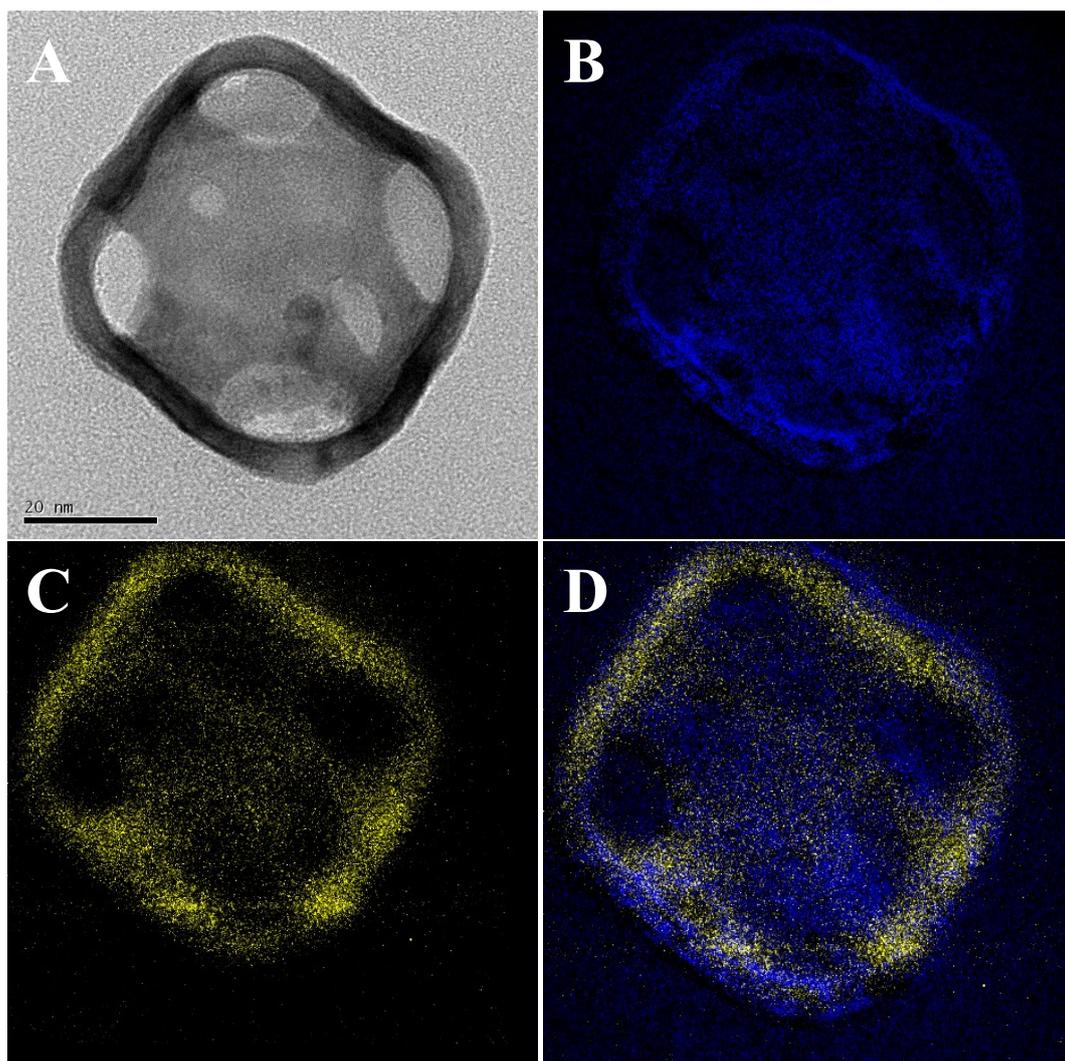


Fig. S6. Element mapping pictures of GNCs. (A) TEM photograph of a GNC particle. (B) and (C) were the gold (yellow) and silver (blue) element distribution in the GNC particle, respectively. (D) The merging picture of plot (B) and (C).

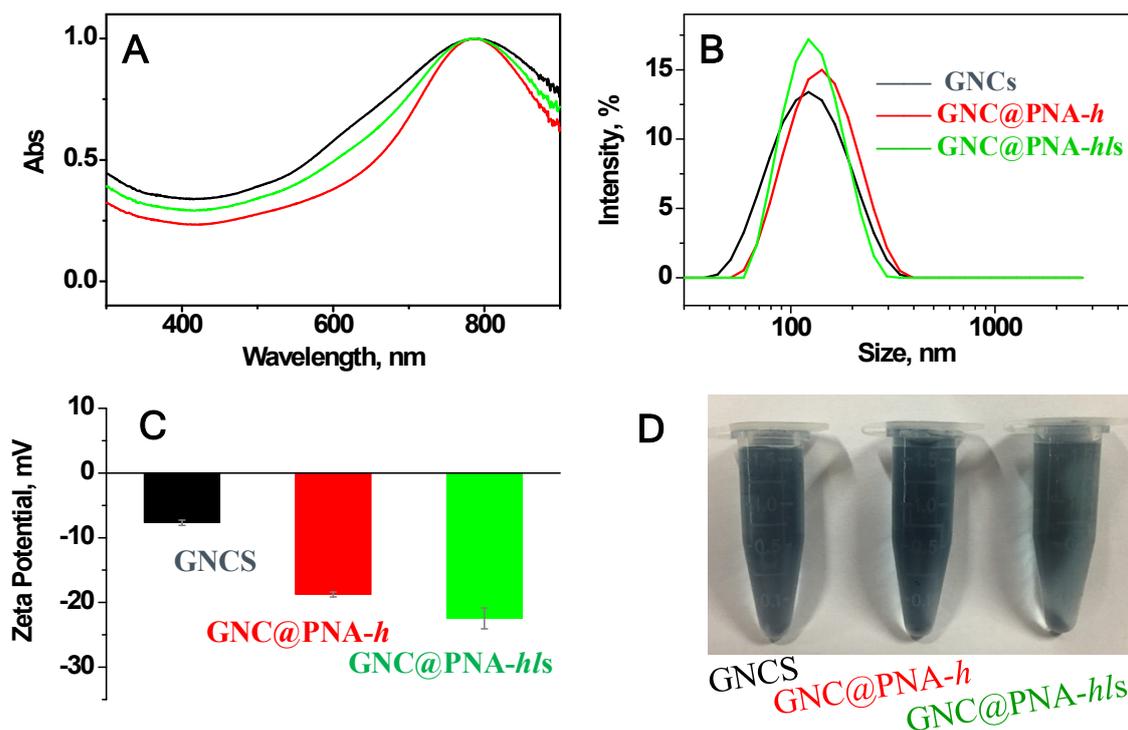


Fig. S7. Physicochemical properties of resultant GNCs. (A) UV/VIS spectrum; (B) The hydrodynamic size measured by dynamic scattering technique; (C) The zeta potentials; (D) photos of various GNCs. GNCs (black), GNC@PNA-*hs* (red) and GNC@PNA-*hls* (green).

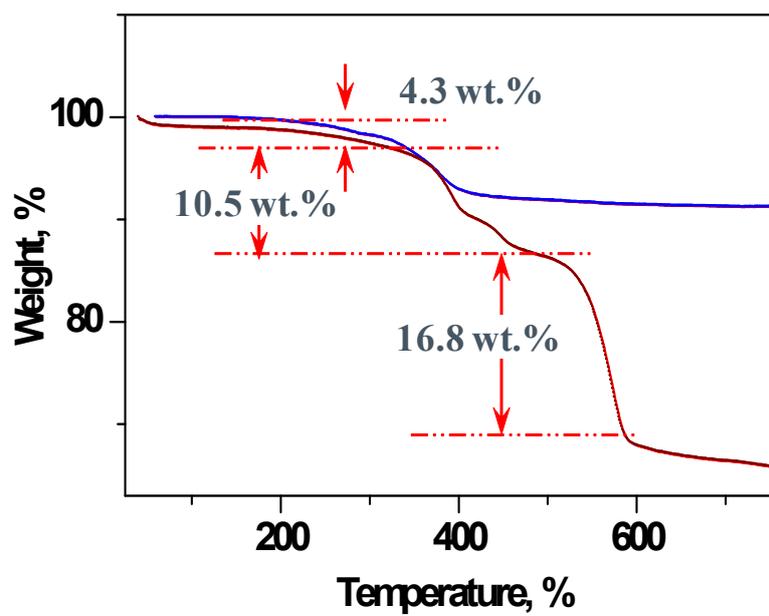


Fig. S8. Thermogravimetric analysis (TGA): GNCs (blue) and GNC@PNA-*hls* (burgundy).

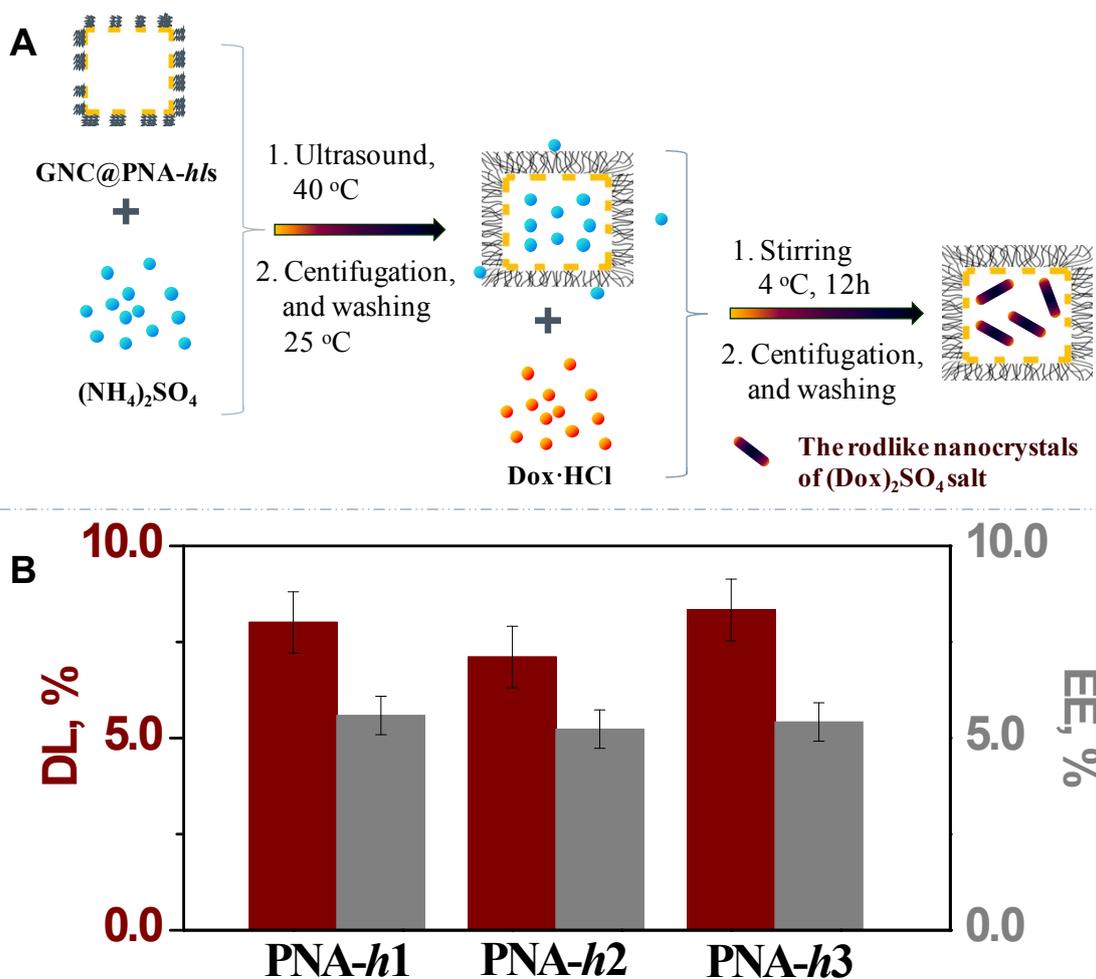


Fig. S9. Doxorubicin loading behavior of GNC@PNA-hls. (A) The schematic illustration of remote loading method with $(\text{NH}_4)_2\text{SO}_4$. (B) Drug-loading amounts (DLs) and entrapment efficiencies (EEs) of GNC@PNA-hls with the modification of various temperature sensitive gated polymers (PNA-h1, PNA-h2 and PNA-h3).

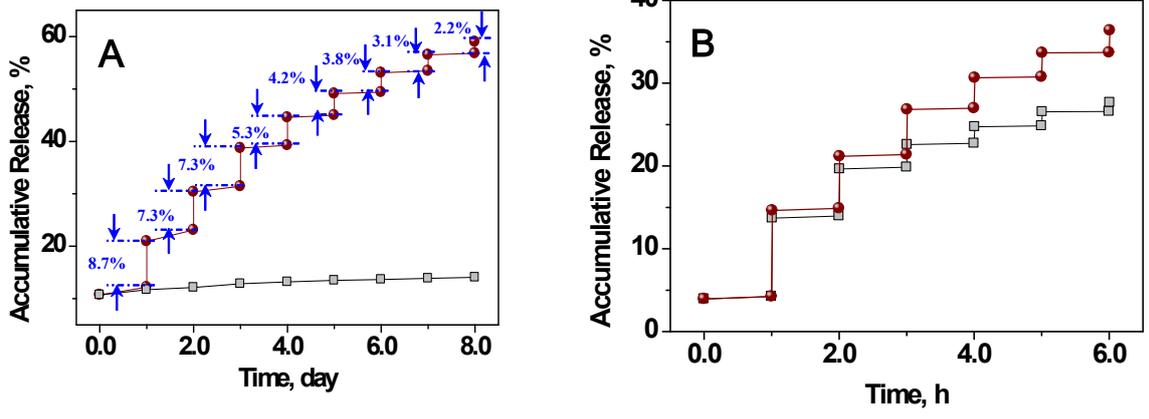


Fig. S10. On-demand release under multiple NIR radiation. (E) Long-term releasing profiles from Dox-GNC@PNA-hls with NIR Laser (burgundy solid sphere) and without NIR laser (black hollow square). $2\text{W}/\text{cm}^2$, 5min. (F) The influence of pH on multiple On-demand release: pH7.4 (black hollow square) and pH6.5 (burgundy solid sphere). $0.4\text{ W}/\text{cm}^2$, 5.0 min.

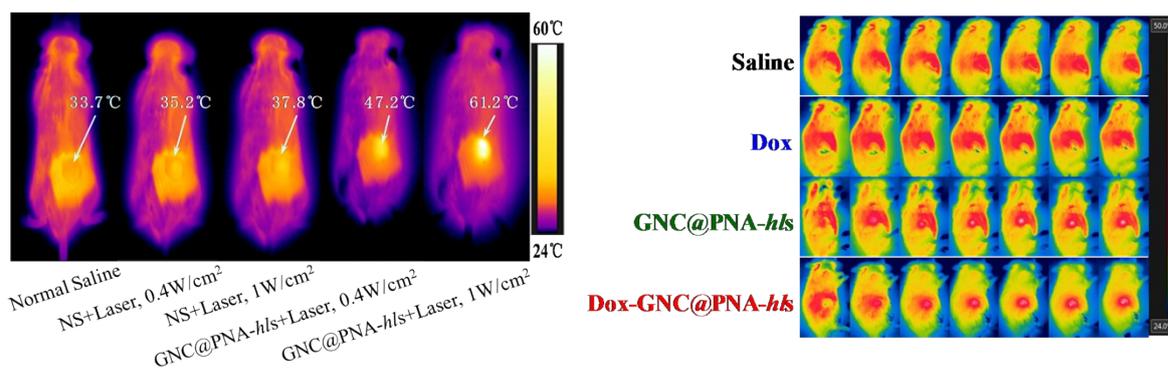


Fig. S11. Thermal imaging comparison of the mice. (A) The influence of laser power on *in vivo* photothermal effect of GNC@PNA-*hls* under NIR radiation, and blank control was normal saline (NS); (B) *in vivo* temperature distribution of various treatments under laser radiation. The dose of Dox and GNCs was 100 $\mu\text{g}/\text{mL}$ and 1000 $\mu\text{g}/\text{mL}$, respectively. Radiation condition: 0.4 W/cm², 5.0 min.

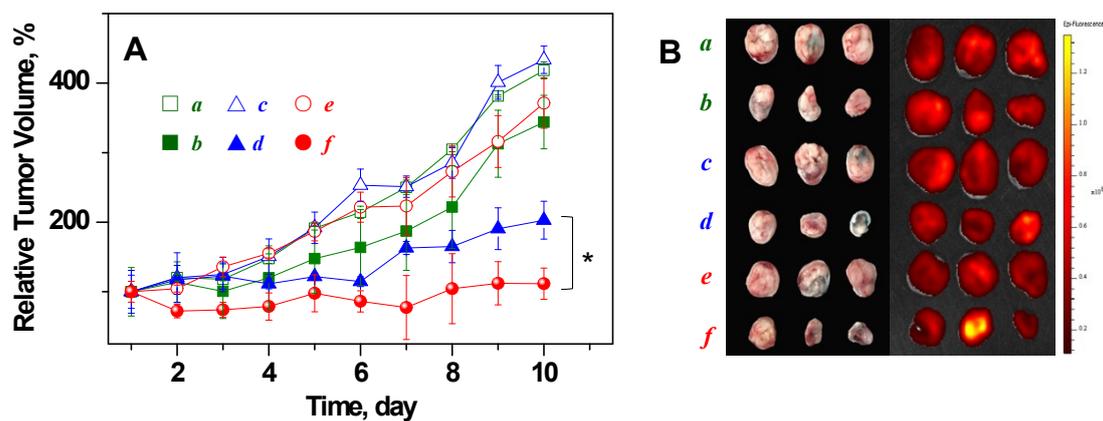


Fig. S12. *In vivo* evaluation on precision synergy between hyperthermia and chemotherapy of Dox-GNC@PNA-*hls*. (A) Tumor growth curves, (B) The gross photos of tumor mass. Group *a* and *b* (green square) were the treatments by the mixing solution of GNC@PNA-*hls* (1000 mg Au/mL) and free doxorubicin (50 μ g/mL) with (solid symbols) and/or without (hollow symbols) NIR radiation (0.4 W, 5.0 min), respectively; Similarly, Group *c* and *d* (blue triangle) were the treatments by the mixing solution of GNC@PNA-*hls* (1000 mg Au/mL) and free doxorubicin (100 μ g/mL) with and/or without NIR radiation, respectively; Group *e* and *f* (red sphere) were the treatments by Do-GNC@PNA-*hls* (1000 mg Au/mL, 100 μ g/mL of doxorubicin) with and/or without NIR radiation, respectively. The injection dosage was 50 μ L per mouse.

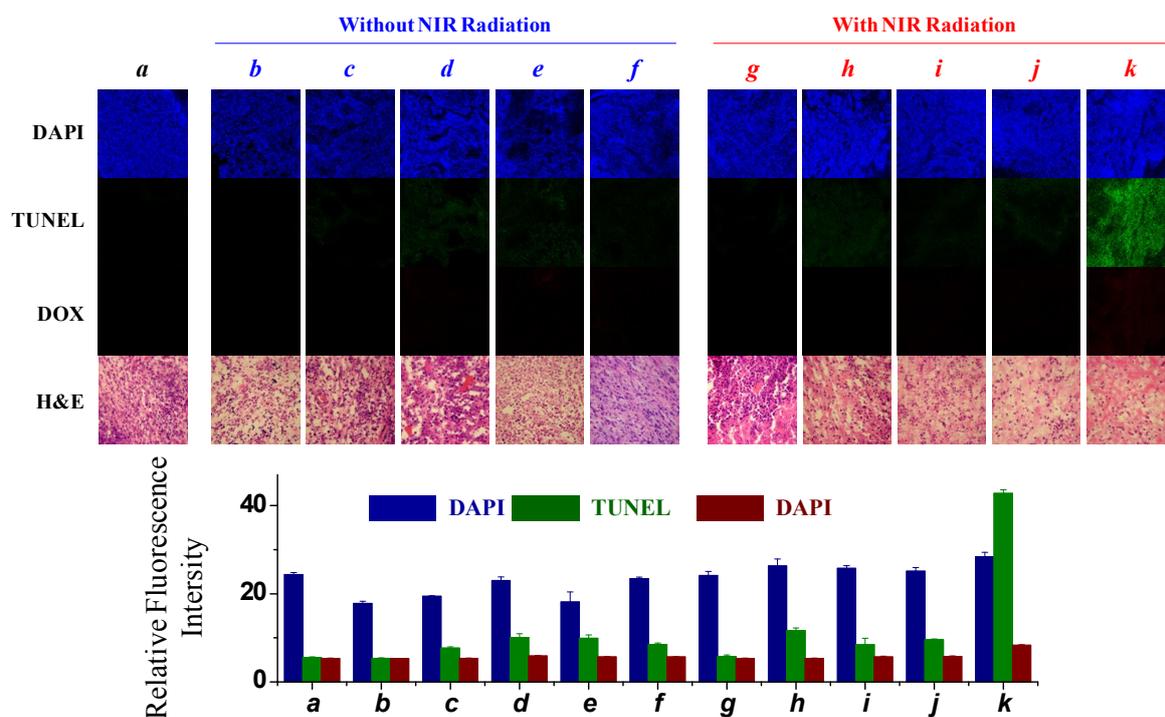


Fig. S13. Immunofluorescence histochemical imaging of H22 tumor slices at 10 days after treatments. The nuclei were stained with DAPI (blue), the apoptosis cells were stained with TUNEL (green). (a) Normal saline; (b) and (g) free dox solution (100 $\mu\text{g}/\text{mL}$); (c) and (h) GNCs (1000 $\mu\text{g}/\text{mL}$); (d) and (i) GNCs + $\frac{1}{2}$ dox: Physical mixture of GNCs and free dox solution. Its concentrations of gold and Dox was 1000 $\mu\text{g}/\text{mL}$ and 50 $\mu\text{g}/\text{mL}$ respectively; (e) and (j) GNCs + dox: Physical mixture of GNCs and free dox solution. Its concentrations of gold and Dox was 1000 $\mu\text{g}/\text{mL}$ and 100 $\mu\text{g}/\text{mL}$ respectively; (f) and (k) Dox-GNC@PNA-*hls*. Its concentrations of gold and Dox was 1000 $\mu\text{g}/\text{mL}$ and 100 $\mu\text{g}/\text{mL}$ respectively. NIR radiation (0.4 W/cm², 5.0 min). The injection dosage was 50 μL per mouse.

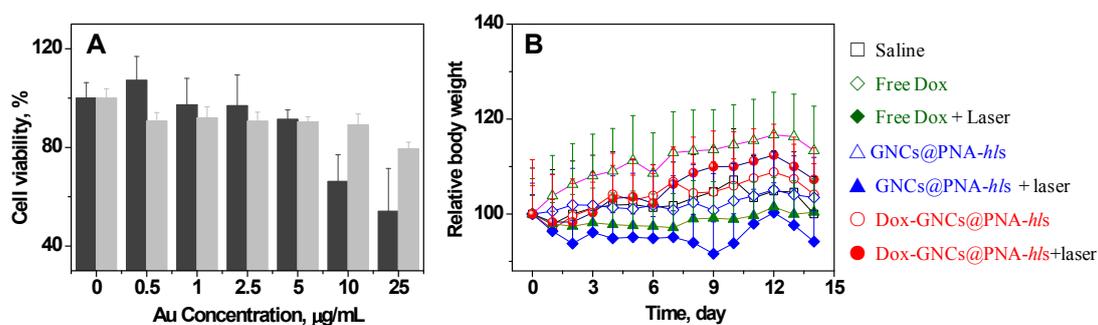


Fig. S14. Biocompatibility of Dox-GNC@PNA-*hls*. A) Cytotoxicities on HepG2 cells (black) and H22 cells (gray) respectively with MTT and CCK8. B) Body weight comparison post various treatments. Saline (black hollow square), Free Dox solution without laser (green hollow diamond) and with laser (green solid diamond), GNC@PNA-*hls* without laser (blue hollow triangle) and with laser (blue solid triangle), Dox-GNC@PNA-*hls* without laser (red hollow sphere) and with laser (red solid sphere). Radiation condition: 0.4 W/cm², 5 min.

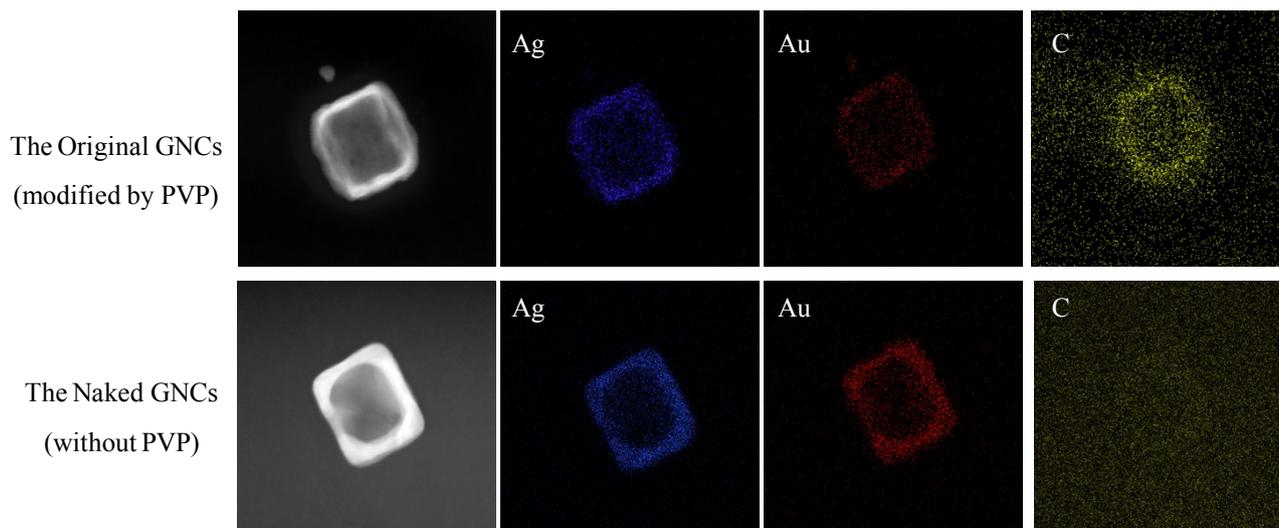


Figure S14. The comparison of element distribution of the original GNCs (before washing with anhydrous alcohol) and the naked GNCs (after washing with anhydrous alcohol)