Supporting Information.

Nanodrug Formed by Co-assembling of Dual Anticancer Drugs to Inhibit Cancer Cell Drug Resistence

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Evaluation of Size and Morphology of Nanostructures. The morphology of HCPT nanorods (NRs) and HD nanoparticles (NPs) was assessed using a scanning electron microscope (SEM, Hitachi S4800). The size distribution and surface charge of HD NPs were measured by dynamic light scattering (DLS, Nano ZS90, Malvern, UK).

Cell Culture. Cells from the human breast cancer line MCF-7R were maintained in Dulbecco's modified Eagle's medium (DMEM) with 4.5 g/L glucose, 10% fetal bovine serum (FBS) and antibiotics (100 units/mL penicillin and 100 μg/mL streptomycin) in a water-jacketed CO_2 incubator (Forma™ Series II 3110, Thermo Fisher Scientific Inc., USA) providing a humidified atmosphere containing 5 % CO_2 at 37 °C.

Colony Formation Assay. Cells were trypsinized and plated at a density of 500 per plate. Fourteen days later, drugs in cell culture medium were added to the plates, and cells were incubated for a further 7 days. Cells were then fixed with 3% paraformaldehyde, stained with crystal violet and imaged with a light microscope. The experiment was performed in triplicate. The number of colonies, defined as containing >50 cells, was counted.

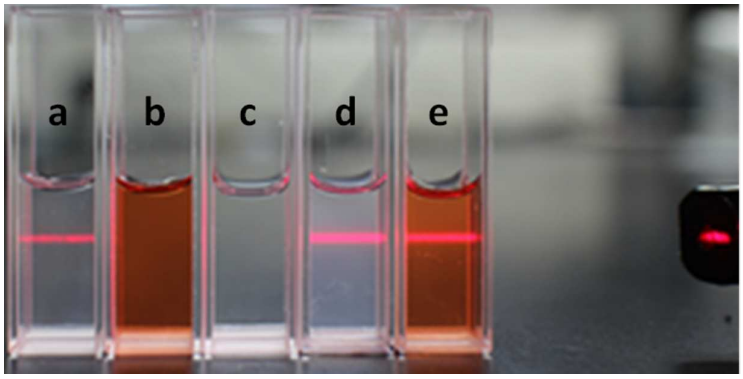


Figure S1. Tyndall effect of a) HCPT in ethanol/water solution (50% water fraction), b) DOX in aqueous solution, c) H_2O, d) HCPT NRs in ethanol/water solution (90% water fraction), e) HD NPs in ethanol/water solution (90% water fraction).

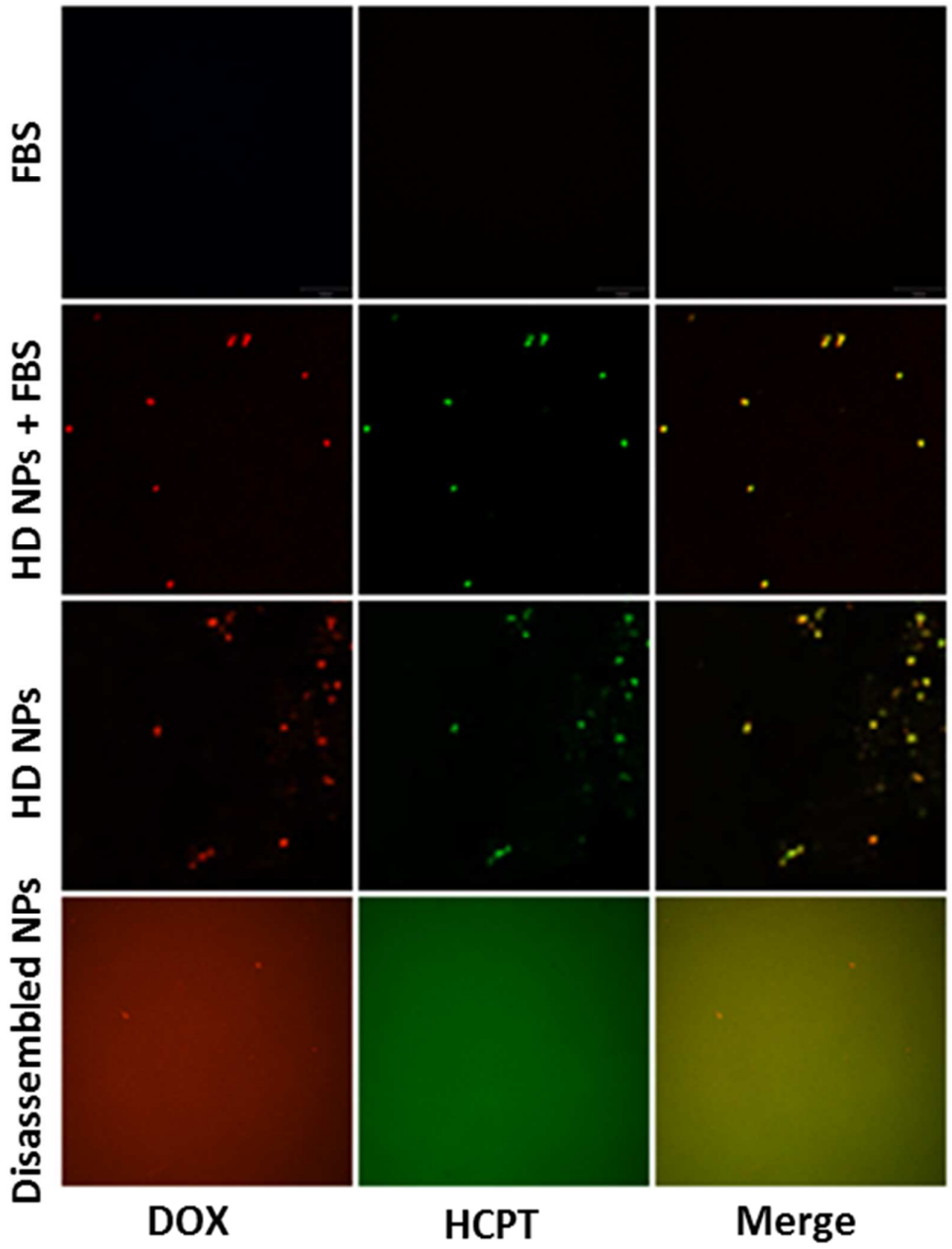
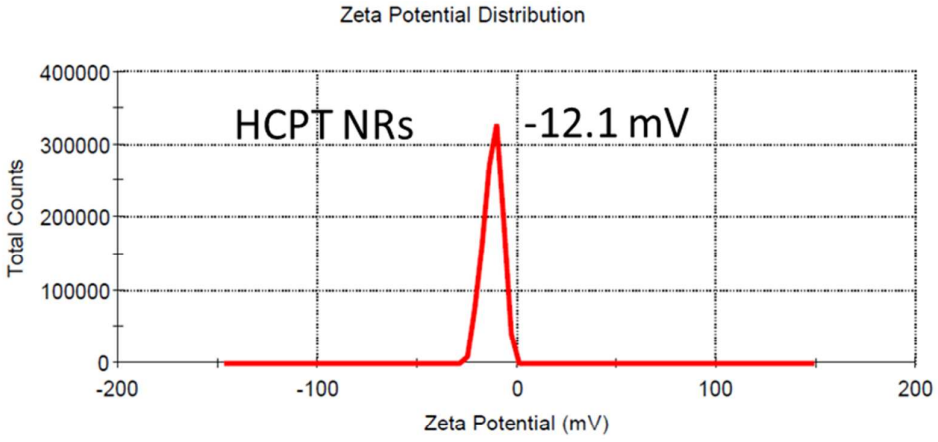


Figure S2. Analysis of the stability of HD NPs dispersed in 10% FBS. Confocal images of FBS solution, HD NPs + 10% FBS solution, HD NPs and dis-assembled HD NPs. The HD NPs were incubated in 10% FBS at 37 °C for 2 hours. The disassembled HD NPs were prepared by adding DMSO to a solution of HD NPs to release the drug molecules.

Results

		Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): -12.1	Peak 1:	-12.1	100.0	4.74
Zeta Deviation (mV): 4.74	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.00260	Peak 3:	0.00	0.0	0.00

Result quality : Good



Results

		Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): 29.1	Peak 1:	29.1	100.0	5.43
Zeta Deviation (mV): 5.43	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.0108	Peak 3:	0.00	0.0	0.00

Result quality : Good

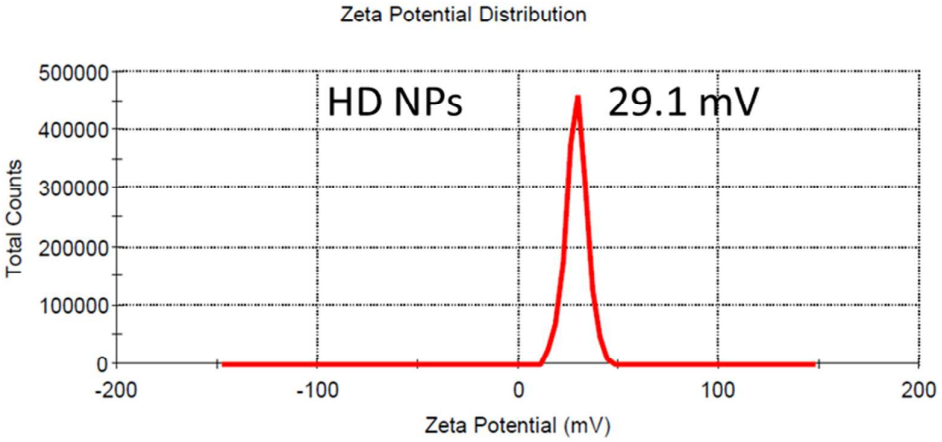


Figure S3. Zeta potential values of HCPT NRs and HD NPs.

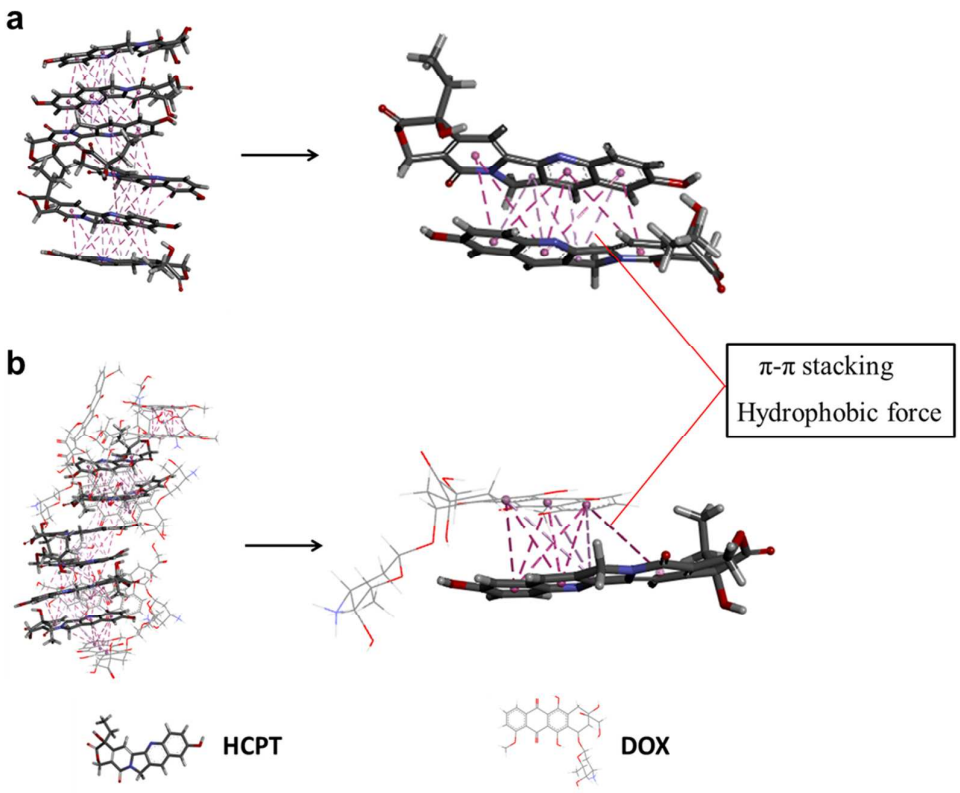


Figure S4. Molecular interaction analysis of a) HCPT self-assembly; and b) HCPT/DOX coassembly by DS 4.0. Hydrophobic forces were shown by purple lines, while π-π stacking interaction was marked by dark purple lines.

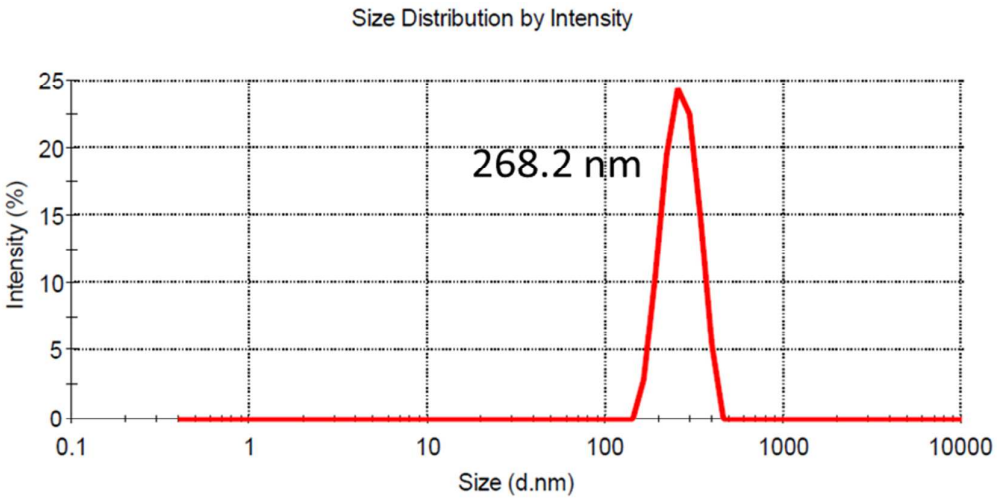


Figure S5. Size distribution of HD NPs after they were lyophilized and redissolved in water. PDI=0.362.

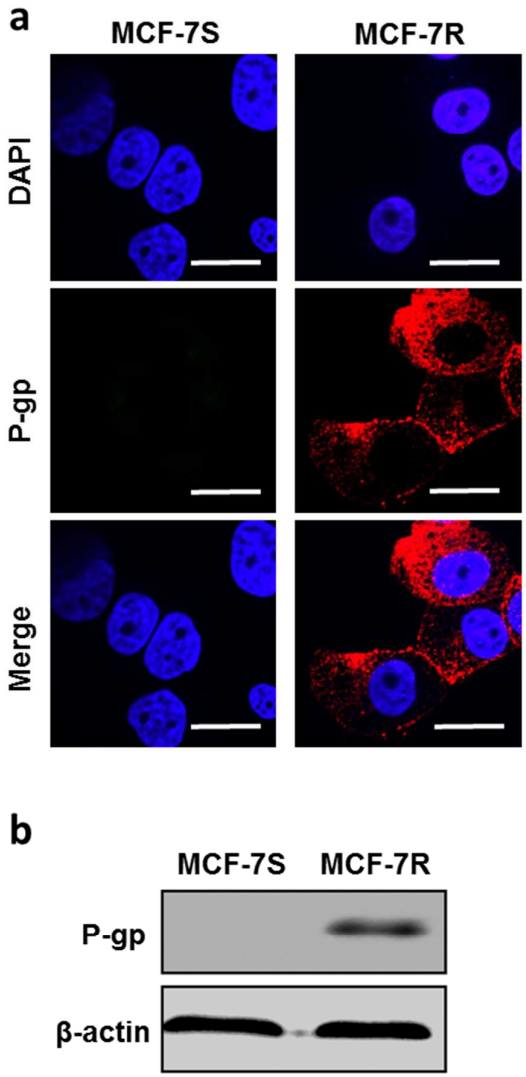


Figure S6. a) Confocal immunofluorescent analysis of MCF-7S (sensitive) and MCF-7R (resistant) cells using MDR1/ABCB1 Rabbit mAb (red). Cell nuclei were stained by Hoechst 33342. Scale bars are 20 μm. b) Western blot of P-gp in MCF-7R and MCF-7S cells.

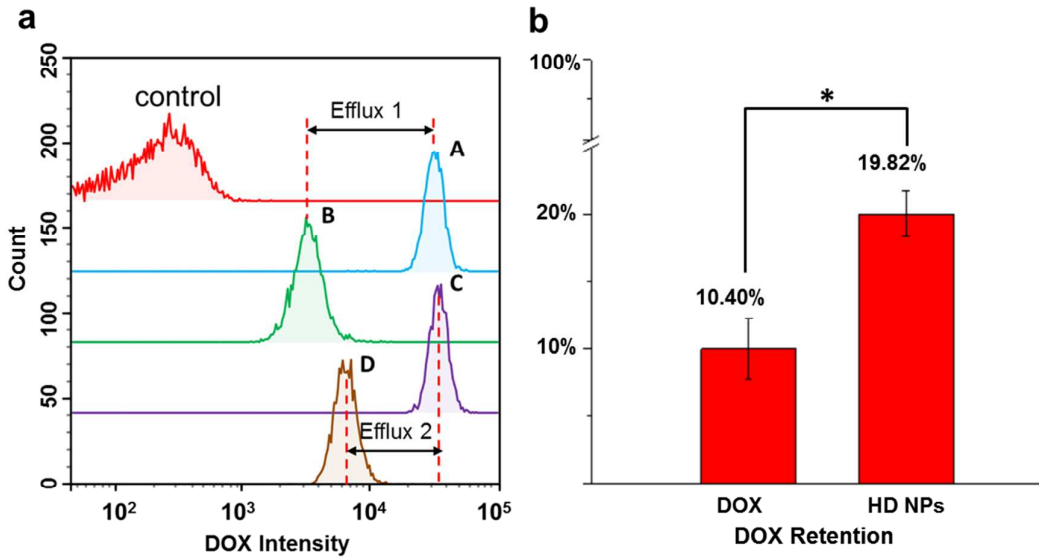


Figure S7. Flow cytometry measurements of P-gp-mediated drug efflux. a) MCF-7R cells were pre-incubated for 1 h with DOX or HD NPs (DOX 40 μM). Half of the cells were analyzed for DOX content (A, free DOX; C, HD NPs). The other half were incubated for another 4 h in drug-free medium, then analyzed for DOX content (B, free DOX; D; HD NPs). DOX efflux was calculated as A-B for free DOX (Efflux 1) and C-D for HD NPs (Efflux 2). Efflux 1 > Efflux 2. b) The percentages of DOX left inner cells after 4h P-gp efflux. The retention percentages was calculated as B/A for free DOX and D/C for HD NPs. *$p<0.05$.