Supplementary information

Self-Assembly Assisted Fabrication of Dextran-Based Nanohydrogels with Reduction-Cleavable Junctions for Applications as Efficient Drug Delivery Systems

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Supplementary information

Materials. Dextran (M_w = 40,000 Da), ceric ammonium nitrate (CAN), methacrylic acid (MAA) and acrylic acid (AA) were purchased from Shanghai Sinopharm Chemical Reagent Co., Ltd. MAA and AA were initially dried over $MgSO_4$ and then distilled under reduced pressure prior to use. CAN was recrystallized from a 3.7 M nitric acid solution containing 11.4 M of ammonium nitrate. Diallyl disulfide (DADS, 80%) was purchased from Sigma Aldrich and used after the removal of impurities (mainly allyl mercaptan) by vacuum distillation. *N,N'*-Methylene bisacrylamide (MBA, Fluka) was recrystallized from methanol. Doxorubicin hydrochloride (DOX, 99%) was purchased from Beijing ZhongShuo Pharmaceutical Technology Development Co., Ltd. L-Glutathione reduced (GSH, ≥98.0%), 4',6-diamidino-2-phenylindole dihydrochloride (DAPI) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma-Aldrich. Dulbecco's modified eagle medium (DMEM) and fetal bovine serum (FBS) were purchased from Hyclone, while penicillin and streptomycin were purchased from Gibco. All other chemical reagents (DMSO, $MgSO_4$, D_2O and deuterated DMSO) were purchased from Shanghai Sinopharm Chemical Reagent Co., Ltd. DMSO was dried over CaH_2 and subsequently distilled under reduced pressure before use. Dialysis membrane bags with a cut-off molecular weight of 14,000 Da were purchased from Shanghai Green Bird Co., Ltd.

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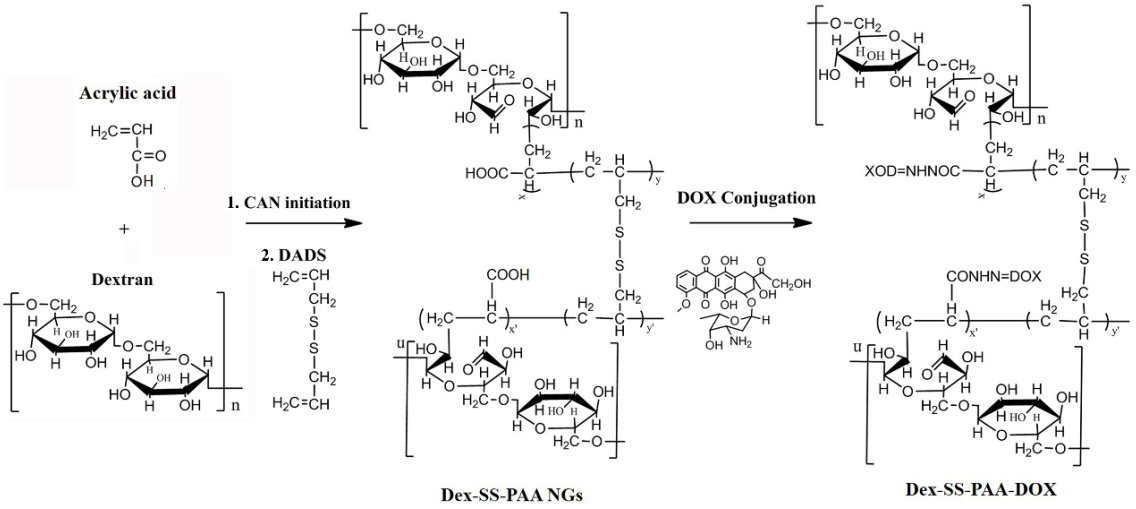

Figure S1. Synthesis of the Dex-SS-PAA and the Dex-SS-PAA-DOX NGs.

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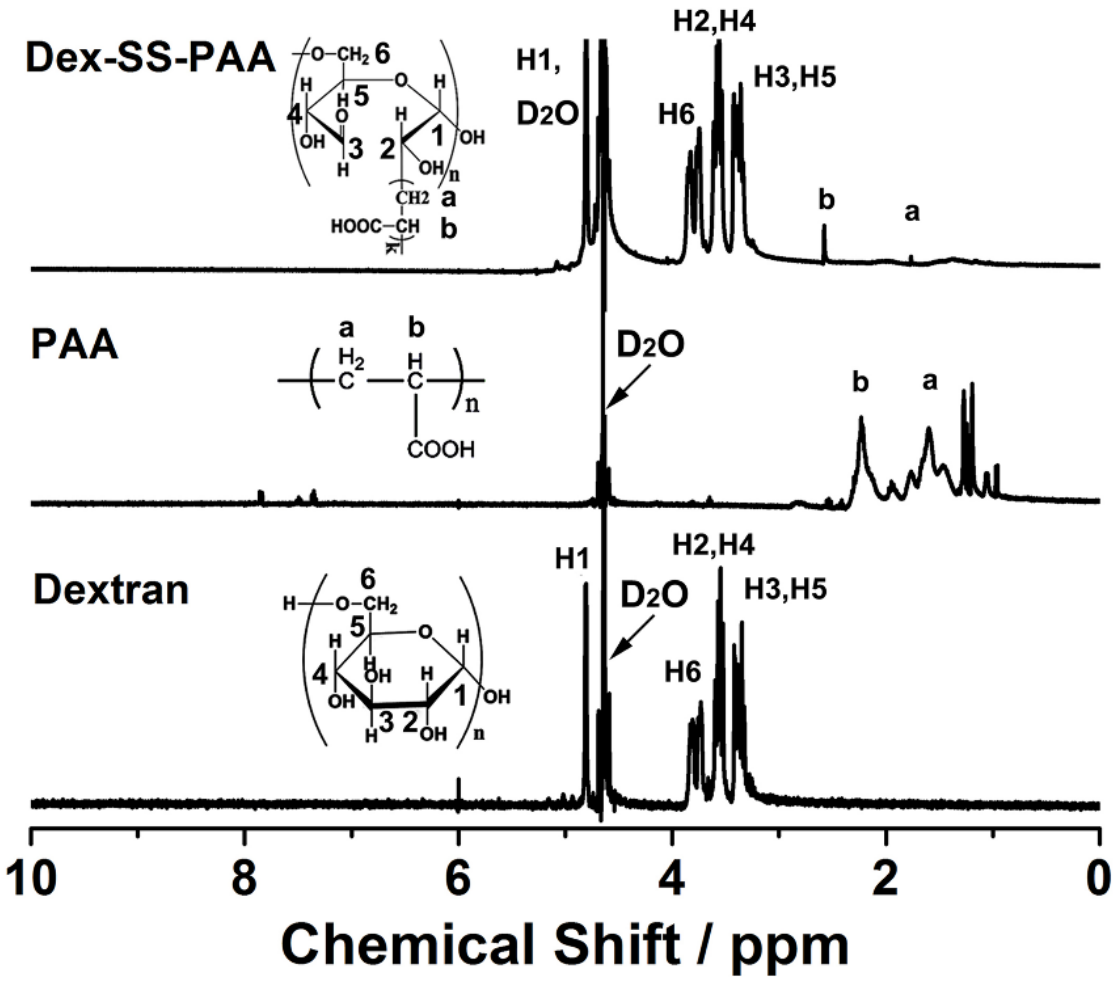

Figure S2. ^{1}H NMR spectra (D_2O) of dextran, PAA and Dex-SS-PAA NGs.

Supplementary information

Table S1. DLS characterization details of Dex-SS-PAA NGs prepared at different AA-to-dextran unit molar ratios.

Sample	$n_{Monomer}$: n_{Glu} [a]	n_{Glu}: $n_{Initiator}$	$n_{Monomer}$: $n_{Crosslinker}$	$<D_h>$ [nm]	PDI	Zeta Potential / mV
Dex-SS-PAA (S)	0.5:1	7:1	10:1	37.5 ± 0.2	0.312	-4.9 ± 1.5
Dex-SS-PAA	1:1	7:1	10:1	98.0 ± 5.0	0.191	-10.0 ± 0.7
Dex-SS-PAA (L)	2:1	7:1	10:1	944.3 ± 66.5	0.805	-7.4 ± 0.3

a) n is the molar amount, $n_{Glu}=[m_{Dex}/M_{wDex}]/M_{wGlu}$, where $M_{wGlu}=168$, m_{Dex} and M_{wDex} are the weight and weight-average molecular weight.

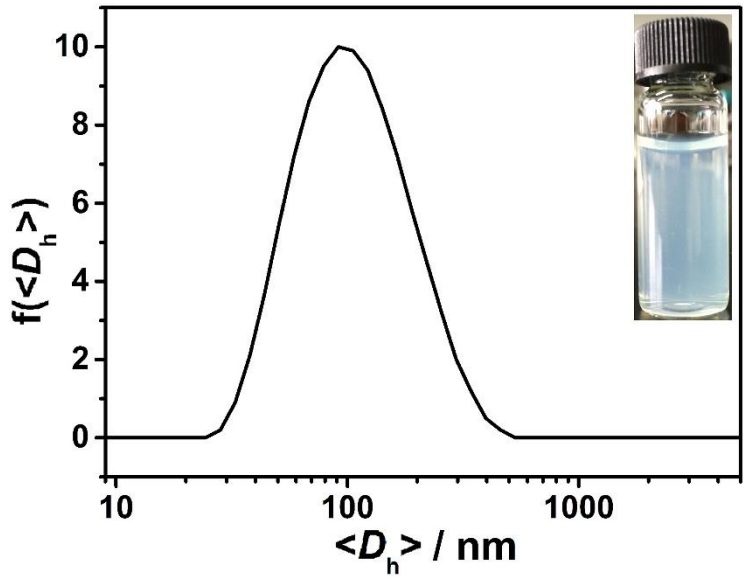

Figure S3. The size distribution of Dex-SS-PMAA NGs ($<D_h>$ = 90.6 nm, PDI = 0.255, zeta potential = -9.5 mV), the inset shows a photograph of the Dex-SS-PMAA NGs aqueous solution at a concentration of 29 g/L.

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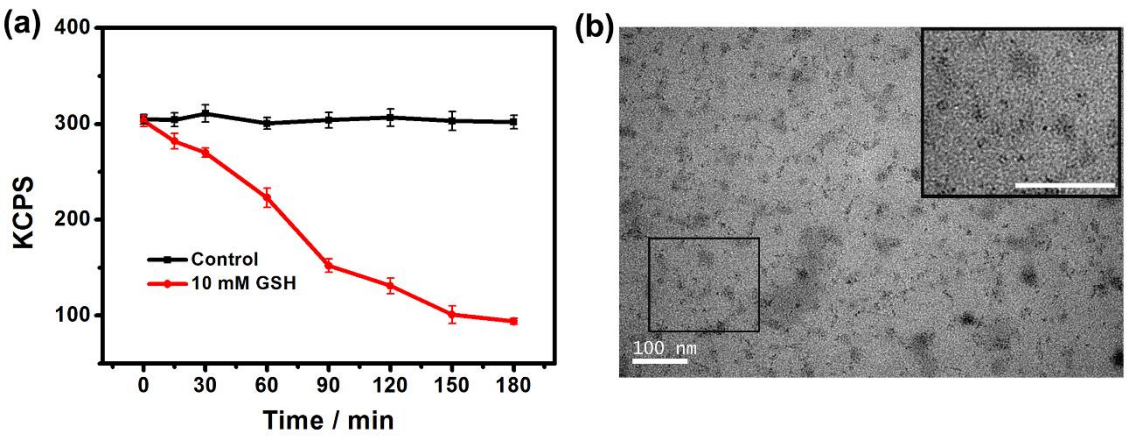

Figure S4. (a) Plots of the kilo counts per second (KCPS) of the Dex-SS-PAA NGs solution after the addition of 10 mM GSH as functions of time. (b) TEM image of Dex-SS-PAA at 30 min after adding GSH. Scale bar: 100 nm in the inset.

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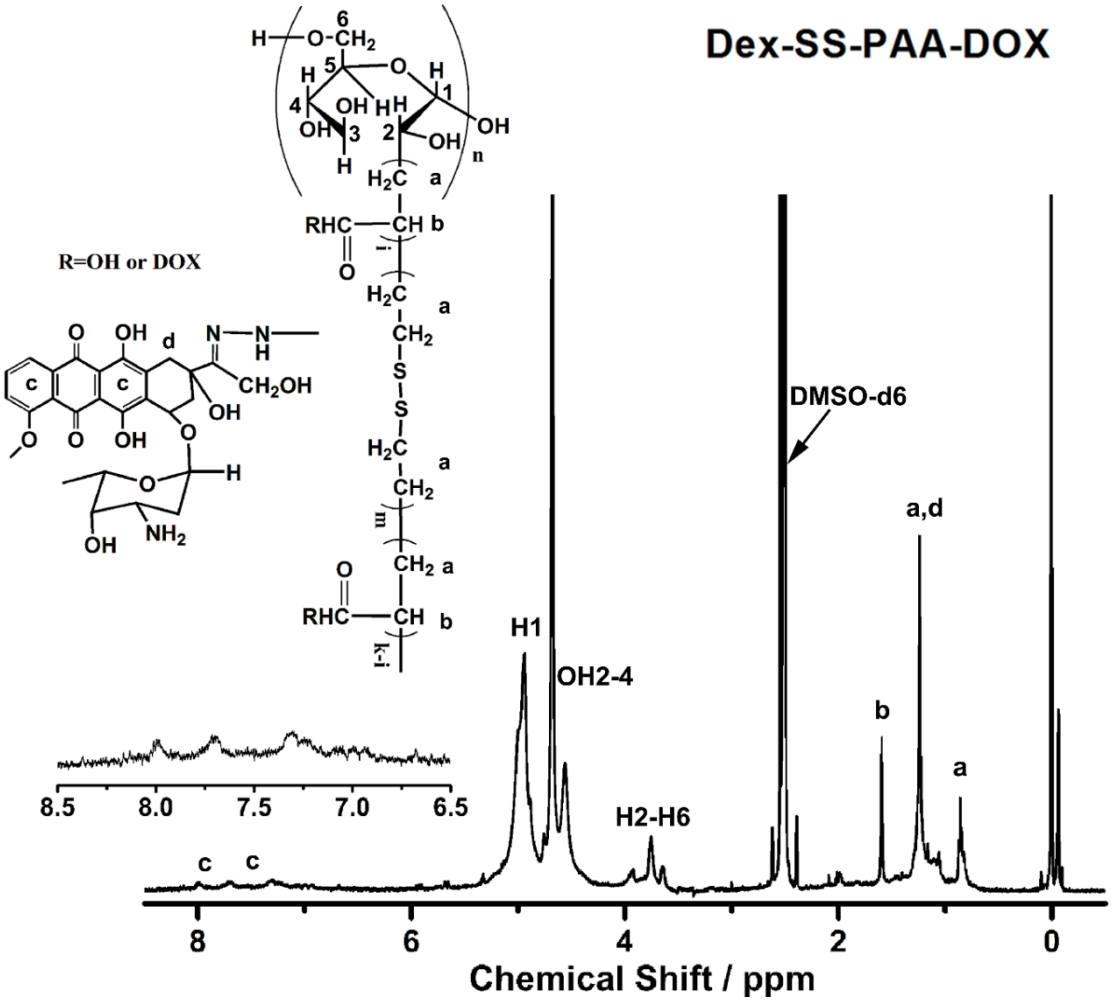

Figure S5. ^{1}H NMR spectrum of Dex-SS-PAA-DOX NGs.

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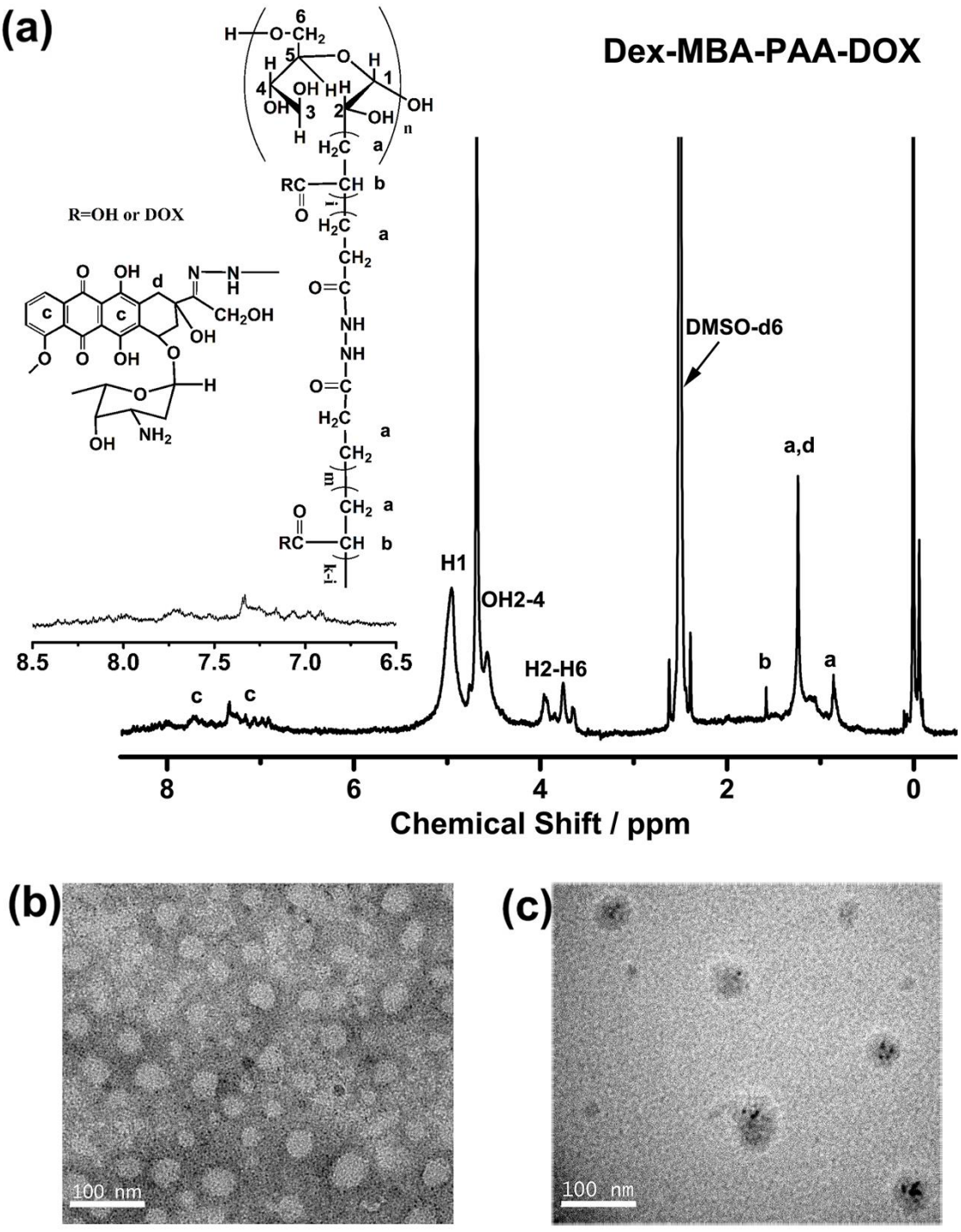

Figure S6. (a) ^{1}H NMR spectrum of Dex-MBA-PAA-DOX. TEM images of (b) Dex-MBA-PAA and of (c) Dex-MBA-PAA-DOX.

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Table S2. DLS measurements of Dex-MBA-PAA NGs and Dex-MBA-PAA-DOX.

Sample	$\langle D_h \rangle$ / nm	PDI	Zeta Potential / mV
Dex-MBA-PAA NGs	107.2 ± 2.6	0.139	-10.1 ± 1.0
Dex-MBA-PAA-DOX	116.4 ± 9.8	0.265	-33.2 ± 2.5

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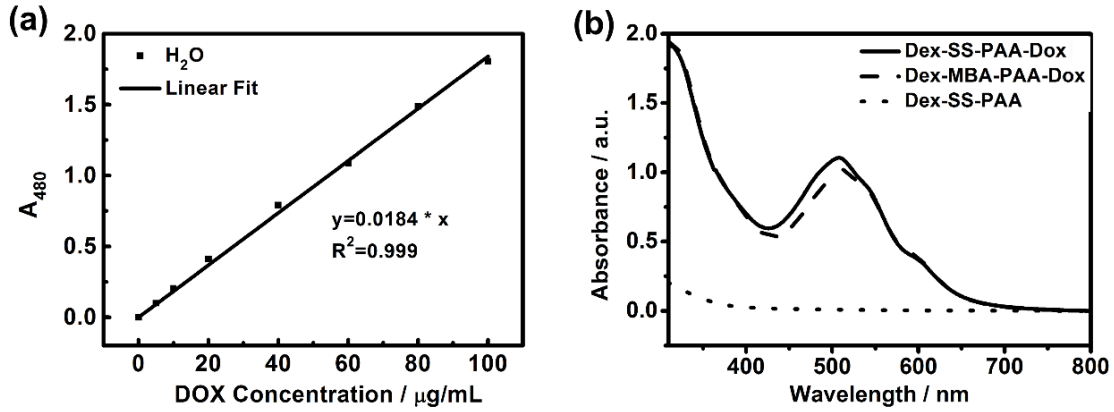

Figure S7. (a) The UV-Vis absorbance calibration curve of various concentrations of DOX solutions at 480 nm. (b) The UV-Vis absorbance spectra of Dex-SS-PAA NGs, Dex-SS-PAA-DOX NGs and Dex-MBA-PAA-DOX NGs (1 mg/mL).

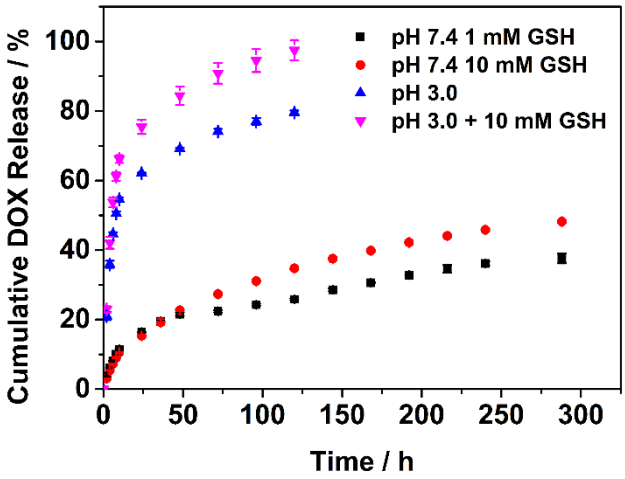

Figure S8. *In vitro* drug release profiles of Dex-SS-PAA-DOX NGs at various GSH concentrations.

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Table S3. pH values of different types of PBS buffers with GSH addition

pH and concentration of phosphate buffer (before GSH addition)	pH 1 mM GSH addition	pH 5 mM GSH addition	pH 10 mM GSH addition
7.4 (0.01 M)	7.1	6.5	5.0
5.0 (0.01 M)	5.0	4.3	3.8
7.4 (0.05 M)	7.2	7.0	6.7
5.0 (0.05 M)	5.0	4.9	4.7

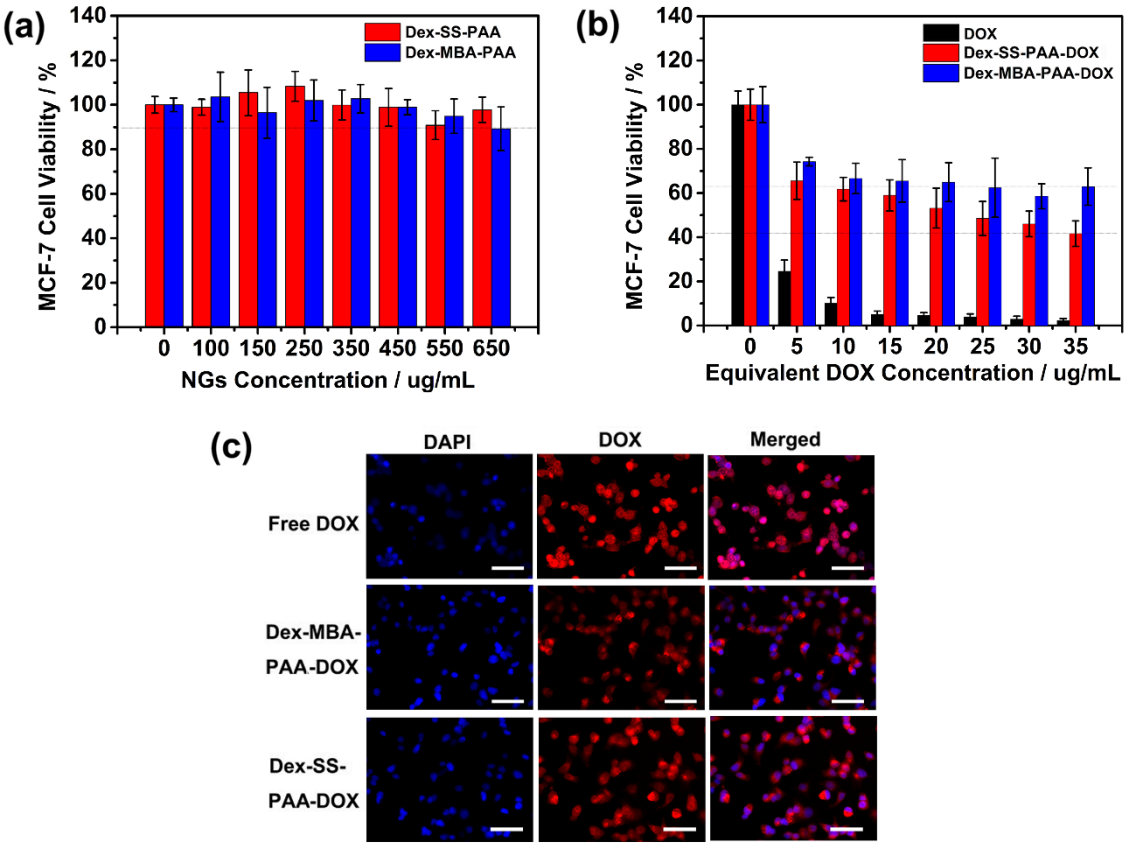

Figure S9. (a) MCF-7 cell viability of non-drug-loaded Dex-SS-PAA NGs and Dex-MBA-PAA NGs. (b) MCF-7 cell inhibition capabilities of free DOX, Dex-SS-PAA-DOX NGs and Dex-MBA-PAA-DOX NGs. (c) Fluorescence microscope images of MCF-7 cells after co-incubation with free DOX, Dex-MBA-PAA-DOX NGs and Dex-SS-PAA-DOX NGs for 48 h at an equivalent DOX concentration of 15 μg/mL. The red colour corresponds to the fluorescence of DOX, while the blue colour corresponds to the fluorescence of DAPI (a cell nucleus dye). Scale bars: 100 μm.