Supporting Information

Microenvironment Responsive DNA-conjugated Albumin Nanocarriers for Targeting Therapy

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Content

S1. Circular Dichroism (CD) Spectroscopy Assay

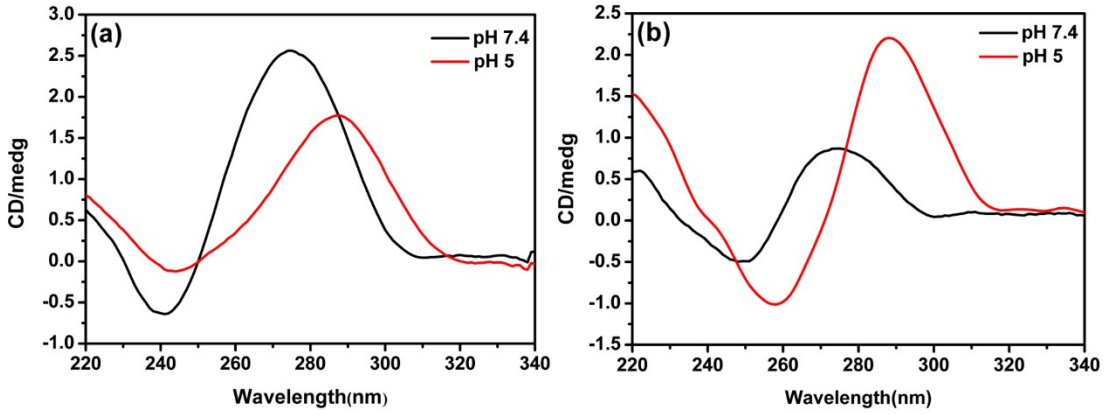
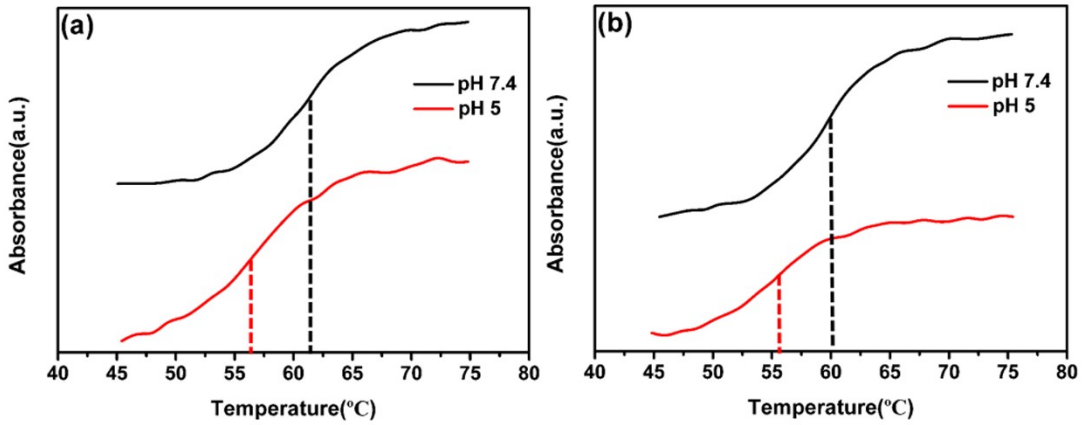


Figure S1. Circular dichroism spectrum of (a) Linker1 and (b) Linker2 in different pH environments.

The pH-responsive drug release is achieved by DNA conformation change. Circular dichroism(CD) spectropolarimetry was adopted to feedback the information about conformation change of Linker1 and Linker2 in different pH environments. As shown in Figure S1, the CD spectrum of Linker1 reveals the negative peak at 240 nm and the positive peak at 276 nm that implies the formation of G-quadruplex conformation contrasted to Linker2, an ordinary single-stranded, which the negative peak at 251 nm and the positive peak at 274 nm in a normal physiological environment (pH 7.4) and the results are consistent with the previous report.[1] As expected, distinct changes emerge when the cultured environment is adjusted to sub-acidity (pH 5). The negative and positive peaks are red-shifted and appear at 244 and 286 nm or 258 and 288 nm for Linker1 and Linker2, respectively, which demonstrate the i-motif structures are successful in formation.[2, 3] Thus, the pH-responsive conformation changes of the nanocarriers not only remained stable in the transmission path under a normal physiology environment but also provided an "OFF-ON" drug delivery performance triggered by passive targeting (e.g., EPR effect), consequently promoted specific uptake into the cancer cells.

S2. The Melting Curves Assay



	pH 7.4	pH 5
T_m(℃)	62.07	56.91

	pH 7.4	pH 5
T_m(℃)	60.32	55.87

Figure S2. Melt curves of (a) Linker1 hybridized dsDNA and (b) Linker2 hybridized dsDNA at pH 7.4 and 5 were determined by following changes in the absorbance at 260 nm.

The melting curves were determined by following changes in the absorbance at 260 nm, and the melting temperatures were obtained by taking the maximum of the first derivative of the curve. The T_m of Linker1 hybridized dsDNA (dsDNA1) and Linker2 hybridized dsDNA (dsDNA2) at pH 7.4 and 5 are shown in Figure S2. At pH 7.4, dsDNA1 displays higher T_m than dsDNA2, which can be attributed to the Linker1 containing more bases. Whereas at pH 5, the T_m of both dsDNA1 and dsDNA2 reduce about five degrees centigrade, which means the thermal stability of samples is inferior to pH 7.4. These two samples' lower T_m at acidic pH confirms the pH-responsive conformation changes and the mechanism triggered by acidic pH. At pH lower than 6.0, the linkers are formed i-motif structure and dehybridized from dsDNA, and simultaneously DBCO-DNA is returned to ssDNA. These results further indicate that the nanocarriers possess the pH-responsive capacity.

S3. Quantitative analysis of DNA grafting onto BSA surface

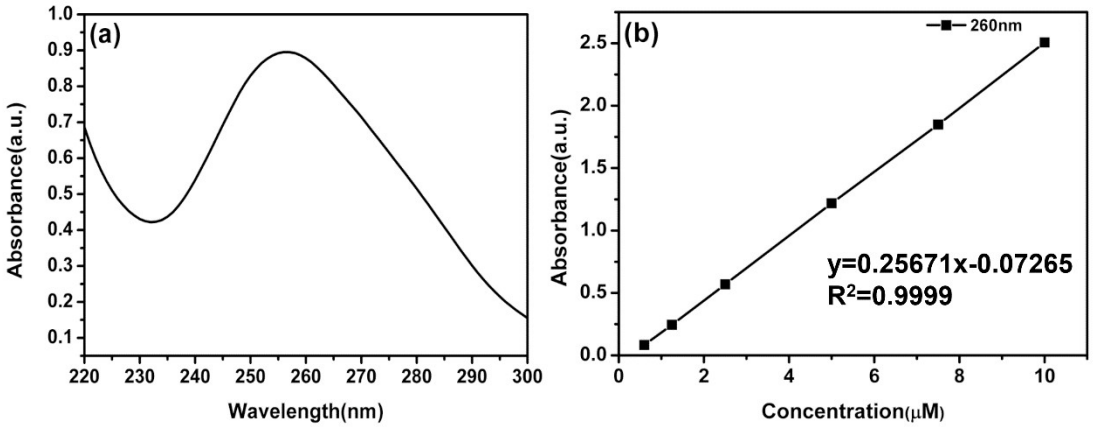


Figure S3. (a) UV–vis absorption spectra of ssDNA-BSA calculated DNA concentration by (b) standard curve of DBCO-DNA between concentration and absorbance at 260 nm.

S4. TEM images of BSA and DNA-BSA@DOX

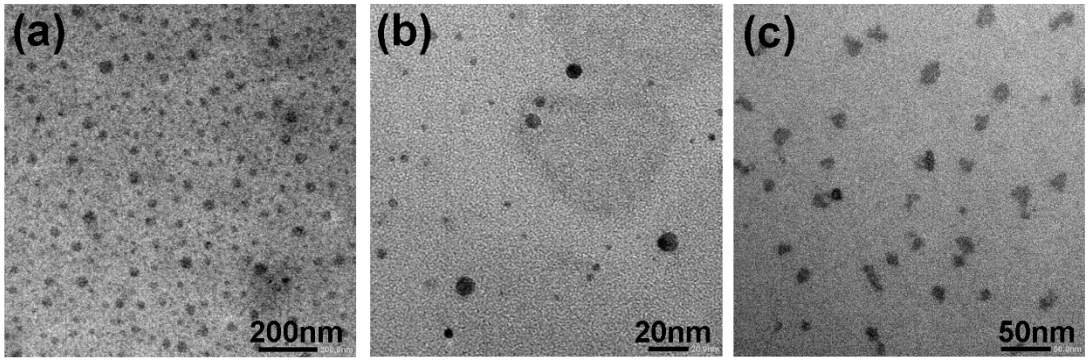


Figure S4. TEM images of (a) BSA, (b) DNA-BSA1@DOX, and (c) DNA-BSA2@DOX.

S5. Fluorescence intensity standard curve of DOX

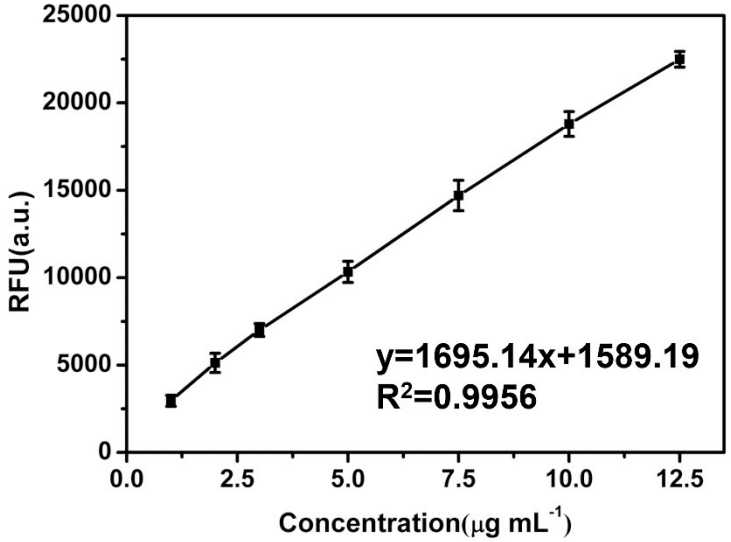


Figure S5. Fluorescence intensity standard curve of DOX with concentration from 1 to 12.5 μg mL^{-1}.

S6. **DOX Encapsulation Efficiency Assay**

To load the drug into the nanocarriers, a mixture of DOX solution (from 20 to 90 μg mL^{-1}) with DNA-BSA solution (1 μM) was reacted at room temperature for 40min. Then, 100 μL of the sample was added to 96-well plates. The fluorescence spectra of DOX (excitation, 480 nm; emission, 595 nm) was measured by a microplate reader (Bio-Tek).

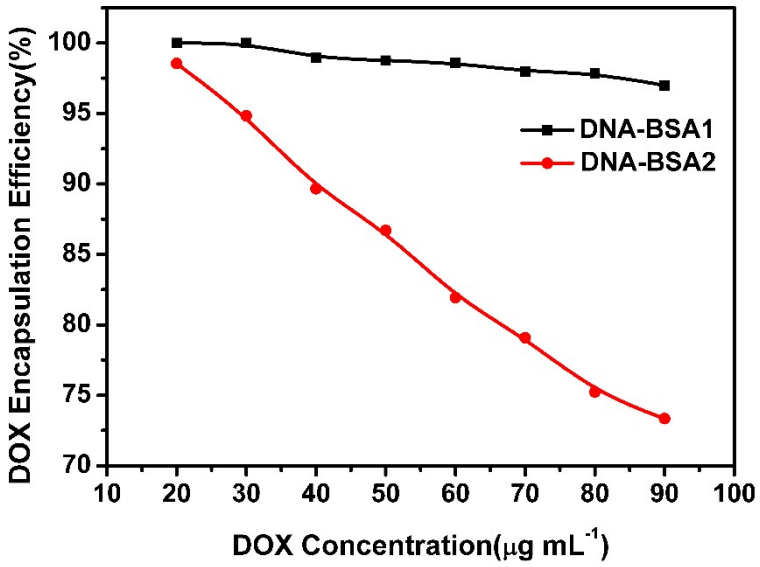


Figure S6. The DOX encapsulation efficiency of DNA-BSA1 and DNA-BSA2 at different feeding concentrations with a constant nanocarriers concentration (1 μM).

S7. Fluorescence spectra of BSA@DOX

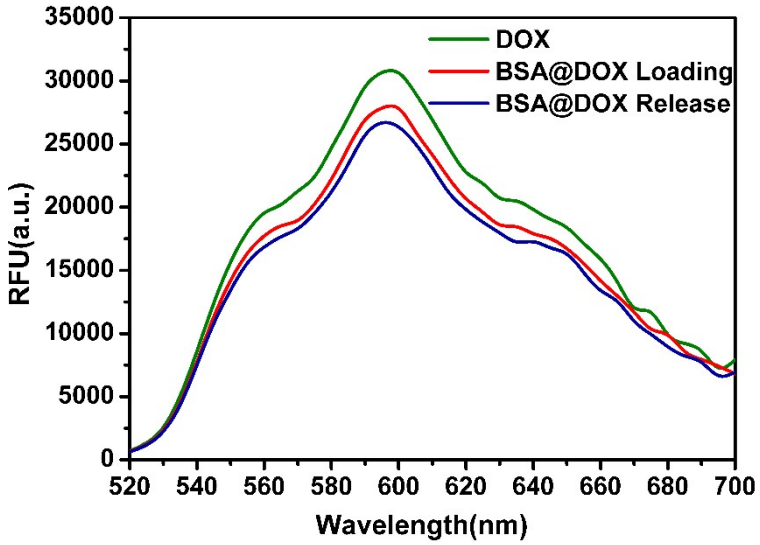


Figure S7. Fluorescence spectra of BSA@DOX loading and release by changing pH value.

S8. Drug loading and release of methylene blue (MB) and daunorubicin (DNR)

To load the drug into the nanocarriers, mixtures of DNA-BSA solution (1 μM) with MB or DNR solution (20 μg mL^{-1}) were reacted at room temperature for 40min. Then, 100 μL of the sample was added to 96-well plates, and the fluorescence spectra (MB: excitation, 600 nm, emission, 695 nm; DNR: excitation, 480 nm, emission, 595 nm) were measured by a microplate reader (Bio-Tek).

To investigate pH-triggered drug release behavior from DNA-BSA nanocarriers, drug-loaded nanocarriers were diluted with PBS buffer (pH 5.0) to final concentration at 500 nM and DOX concentration at 10 μg mL^{-1}. After that, incubated at 37 °C, the released DOX was recorded by the above-mentioned method.

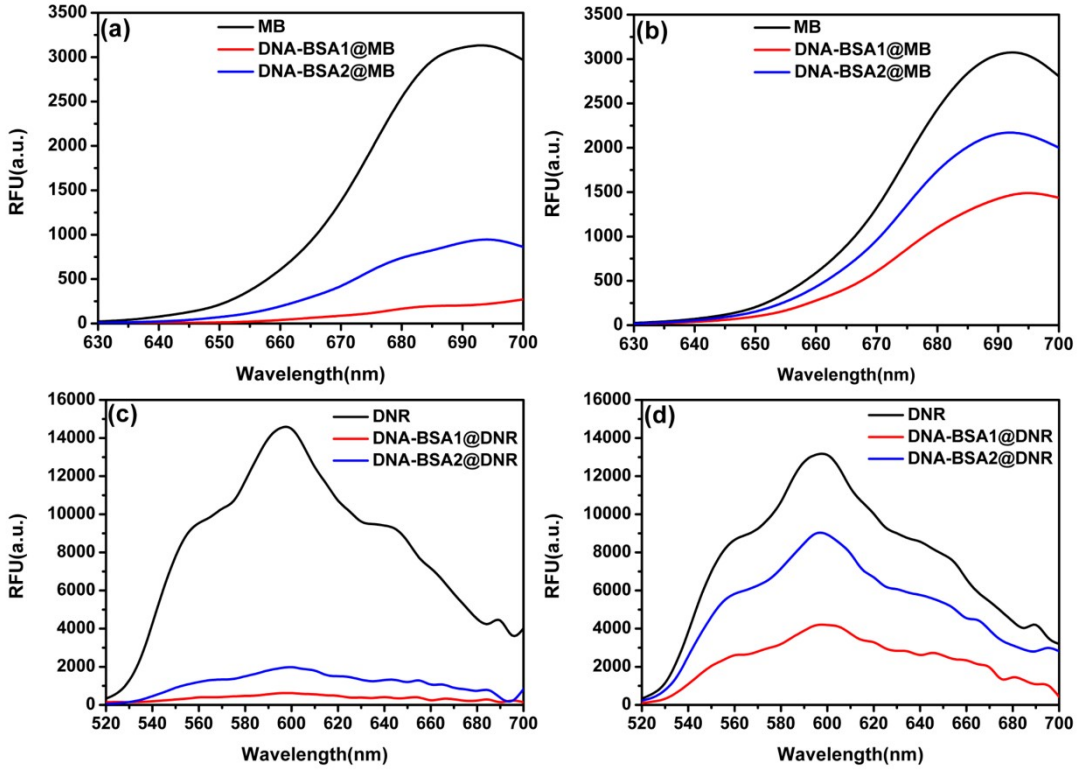


Figure S8. Fluorescence spectra of MB (a) or DNR (c) loading into DNA-BSA1 and DNA-BSA2 and release (b) or (d) under acid condition.

S9. Hemocompatibility Assay

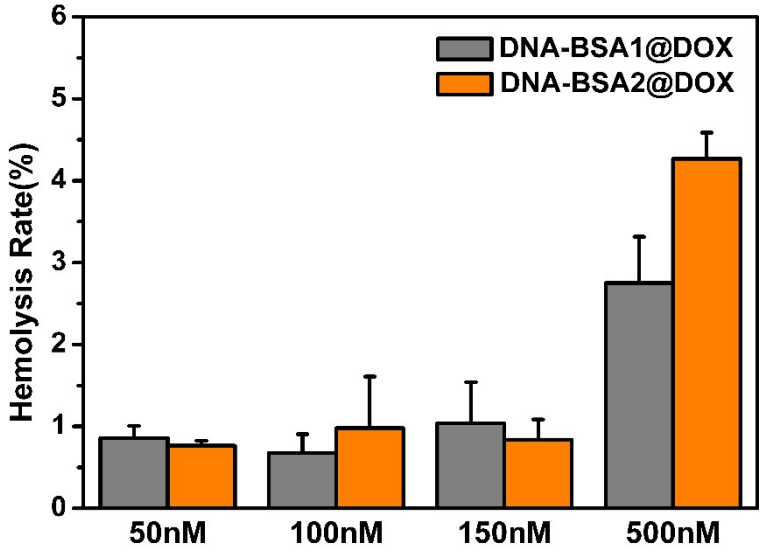


Figure S9. Hemolysis rates of DNA-BSA@DOX with different concentrations.

Outstanding biocompatibility and hemocompatibility are the important characteristics of biomedical nanomaterials. Thus we performed a hemolysis analysis to evaluate as-prepared nanocarriers. As shown in Figure S7, nanocarriers with concentrations from 0-500 nM do not display an obvious hemolytic effect (< 5%) and correspond to threshold values reported in the previous literature.[4] Thus, DNA-BSA1@DOX and DNA-BSA2@DOX display excellent biocompatibility, on this premise, provide a great guarantee for cancer treatments.

S10. References

1. A. Rajendran and B. U. Nair, *Biochimica Et Biophysica Acta-General Subjects*, 2006, **1760**, 1794-1801.
2. J. Choi, S. Kim, T. Tachikawa, M. Fujitsuka and T. Majima, *Journal of the American Chemical Society*, 2011, **133**, 16146-16153.
3. A. Rajendran, S.-i. Nakano and N. Sugimoto, *Chemical Communications*, 2010, **46**, 1299-1301.
4. Q. Chen, H. Wang, H. Liu, S. Wen, C. Peng, M. Shen, G. Zhang and X. Shi, *Analytical Chemistry*, 2015, **87**, 3949-3956.