Supporting Information for

Conformational Switch-Mediated Accelerated Release of Drug from Cytosine-Rich Nucleic Acid-Capped Magnetic Nanovehicles

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Part I: Materials and Methods

Chemicals: Ferric chloride ($FeCl_3 \cdot 6H_2O$), ferrous chloride ($FeCl_2 \cdot 4H_2O$), oleic acid, ammonium hydroxide aqueous solution (NH_4OH), ethanol (EtOH) and toluene were purchased from Beijing Chemicals. N-cetyltrimethyl-ammonium bromide (CTAB) and fluorescein isothiocyanate (FITC) were obtained from Alfa Aesar. Tetraethyl orthosilicate (TEOS), sodium hydroxide (NaOH), 3-aminopropyl trimethoxysilane (APTES), fluorescein sodium, and doxorubicin hydrochloride (Dox) were obtained from Sigma–Aldrich. Deoxynuclease I was obtained from Promega. All chemical agents were of analytical grade and used directly without further purification. Water throughout all experiments was obtained by using a Milli-Q water system. The oligonucleotides used in the present study were purchased by Sangon Biotechnology Inc.. The sequences are as follows:

C-rich DNA: 5'-CCC TAA CCC TAA CCC TAA CCC-3'

control DNA: 5'-CTC TCA CTC TCA CTC TCC ACC-3'

fluorescent C-rich DNA: 5'-FAM-CCC TAA CCC TAA CCC TAA CCC-3'

Oleate-Modified Magnetic Nanoparticles: $FeCl_3 \cdot 6H_2O$ (100 mmol) and $FeCl_2 \cdot 4H_2O$ (50 mmol) were dissolved in pure water (250 mL), followed by adjusting the solution temperature to 90 ºC. NH_4OH (75 mL) and oletic acid (5 g) were sequentially added rapidly while stirring was continued. Above mixture was maintained for 3 h to give rise to black precipitates. The product was separated with an assistant magnet, washed with water and EtOH, and dried in vacuum overnight. After drying, the oleate-capped magnetic nanoparticles were dispersed in chloroform for further use.

Amido-Decorated Magnetic Nanovehicles: Typically, CTAB (0.8 g) and NaOH (0.2 g) were dissolved in pure water (350 mL) and heated to 80 ºC. Magnetic nanoparticles

in chloroform (500 mg) were poured into the solution while stirring was continued to form a homogeneous oil-in-water microemulsion. To further evaporate chloroform, the resulting solution was vigorously stirred for another 0.5 h. Then, TEOS (4 mL) was added into above mixture under vigorous stirring. The precipitate was collected by centrifugation and rinsed with water and EtOH. To prepare amino-decorated magnetic nanovehicles, above nanoparticles (0.8 g) was refluxed with anhydrous toluene (60 mL) containing APTES (0.5 mL) at 110 ºC for 24 h. The obtained sample was collected and rinsed with EtOH. To further remove the surfactant template of CTAB, the crude product was refluxed with EtOH (200 mL, 95%) containing NH_4NO_3 (500 mg) at 80 ºC for 6 h. The resulting product was washed extensively with EtOH under high vacuum, dried in vacuum over night.

Dox Loading and DNA Capping: Amido-decorated magnetic nanovehicles (20 mg) was soaked in Tris buffer (20 mM, 100 mM NaCl, 10 mL, pH 7.4) containing Dox (1 mg) for 24 h, followed by the addition of excessive DNA (1 mmol). Above suspension was stirred at 37 ºC for 2 h to form Dox-loaded DNA-capped magnetic nanovehicles. These nanocomposites were then washed thoroughly with neutral Tris buffer (5 mL) several times to remove the residual Dox and the free DNA absorbed on the nanocomposites. The loading amount of Dox was determined according to the initial and the residual Dox in the buffer system by measuring its absorbance at 486 nm. To investigate the capping efficiency of DNA molecules on the surfaces of magnetic nanovehicles, similar process was carried out without the addition of Dox.

Controlled Release Studies: For different pH values, Dox-loaded DNA-capped magnetic nanovehicles (3 mg) were suspended in Tris buffer (20 mM, 5mL, 100 mM NaCl, pH 7.4) and MES buffer (20 mM, 5 mL, 100 mM NaCl, pH 5.0) with stirring at 37 ºC, respectively. The aliquot incubation medium was taken out for analysis at

every given time intervals, and replaced with the same volume of fresh buffer. The extracted medium was monitored via the fluorescence of Dox. For DNase-responsive experiments, Dox-loaded DNA-capped nanocomposites (3 mg) were suspended in Tris buffer (20 mM, 5mL, 100 mM NaCl, pH 7.4) containing DNase with stirring at 37 ºC. Moreover, control experiments were performed by changing the DNA sequence during the capping and altering the pH value of the buffer system.

Fluorescein Sodium Loading, DNA Capping, and Controlled Released Studies: Amido-decorated magnetic nanovehicles (20 mg) was soaked in Tris buffer (20 mM, 100 mM NaCl, 10 mL, pH 7.4) containing fluorescein sodium (2 mg) for 24 h, followed by the addition of excessive DNA (1 mmol). Above suspension was stirred at 37 ºC for 2 h to form fluorescein sodium-loaded DNA-capped nanovehicles. These nanocomposites were washed thoroughly with neutral Tris buffer to remove the residual fluorescein sodium and the free DNA absorbed on the nanocomposites. The loading amount of fluorescein sodium was determined according to the initial and the residual ones in the buffer system via their fluorescence at 515 nm. For different pH values, fluorescein sodium-loaded DNA-capped nanovehicles (3 mg) were suspended in Tris buffer (20 mM, 5mL, 100 mM NaCl, pH 7.4) and MES buffer (20 mM, 5 mL, 100 mM NaCl, pH 5.0) with stirring at 37 ºC, respectively. For DNase-responsive experiments, fluorescein sodium-loaded DNA-capped nanocomposites (3 mg) were suspended in various buffer containing DNase with stirring at 37 ºC.

Cell Culture: Hela cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), penicillin (100 U/mL) and streptomycin (100 U/mL) in a humidified incubator (37 ºC, 5% CO_2). Cells were harvested by the use of trypsin and were resuspended in fresh complete medium before plating.

Imaging of Cellular Uptake: In a typical experiment, Hela cells with a density of 2 × 10^4 were plated in a 12-well plate for 12 h to allow the attachment of cells. Then, DNA-capped FITC-modified magnetic nanovehicles were added into above medium with a concentration of 200 μg/mL. Upon different incubation conditions, cells were washed with cool 0.9% NaCl solution and treated with LysoTracker Red for hald an hour. At last, fluorescence images were captured using an Olympus BX-51 optical equipped with a CCD camera.

Flow Cytometer Analysis: DNA-capped FITC-modified magnetic nanovehicles and FAM-decorated DNA-capped magnetic nanovehicles were rationally prepared by using FITC-modified APTES and FAM-decorated ssDNA as fluorescence groups, respectively. Then, classical flow cytometer was applied to detect the efficacy of cellular uptake upon different treatments.

Cytotoxicity Studies: Hela cells were cultured in 96-well plates with a density of 5× 10^3 per well for 12 h to allow the attachment of cells. Subsequently, Dox and Dox-loaded DNA-capped magnetic nanovehicles with expected concentrations were added into above culture medium. 6 h after incubation, above medium was removed and changed. Another 24 h later, above medium was removed, and cell samples were treated with MTT for another 4 h, which was followed by the addition of dimethyl sulfoxide (DMSO) to dissolve the formazan crystals. A Bio-Rad model-680 microplate reader was applied to measure the absorbance at a wavelength of 570 nm. Six replicates were done for each treatment group and percent viability was normalized to cell viability without any treatment.

Imaging of Live/Dead Stained Cells and Trypan Blue Stained Cells: Hela cells were incubated with Dox-loaded DNA-capped magnetic nanovehicles (200 μg/mL) for 6 h upon an assistant magnet. Then, the cells were stained with calcein AM and

propidium iodide. The fluorescence microscopy images were collected on an Olympus BX-51 optical system. In addition, trypan blue solution (0.4%) was applied to distinguish live/dead cells under an optical microscope.

Apoptosis and Necrosis Analysis: Hela cells were incubated with Dox, DNA-capped magnetic nanovehicles, and Dox-loaded DNA-capped magnetic nanovehicles to achieve the percentage of apoptosis and necrosis. The concentration of drug used in the group of Dox-loaded DNA-capped magnetic nanovehicles was as same as the group of Dox (2 μg/mL). The concentration of DNA-capped magnetic nanovehicles was calculated to be 135 μg/mL. 12 h after incubation, Annexin V-FITC apoptosis detection kit was used to detect the percentage of apoptosis and necrosis. Then, related data were quantified by fluorescence-activated cell sorting flow cytometry.

Intracellular Fluorescence Imaging of Dox: Hela cells were incubated with Dox-loaded DNA-capped magnetic nanovehicles (200 μg/mL). At each end of the incubation period, the fluorescence microscopy image was collected on an Olympus BX-51 optical system, respectively.

Statistical analysis: All data are expressed herein as mean result ± standard deviation (SD). The statistical analysis was performed by using Origin 8.0 software.

Part II: Figures and Tables

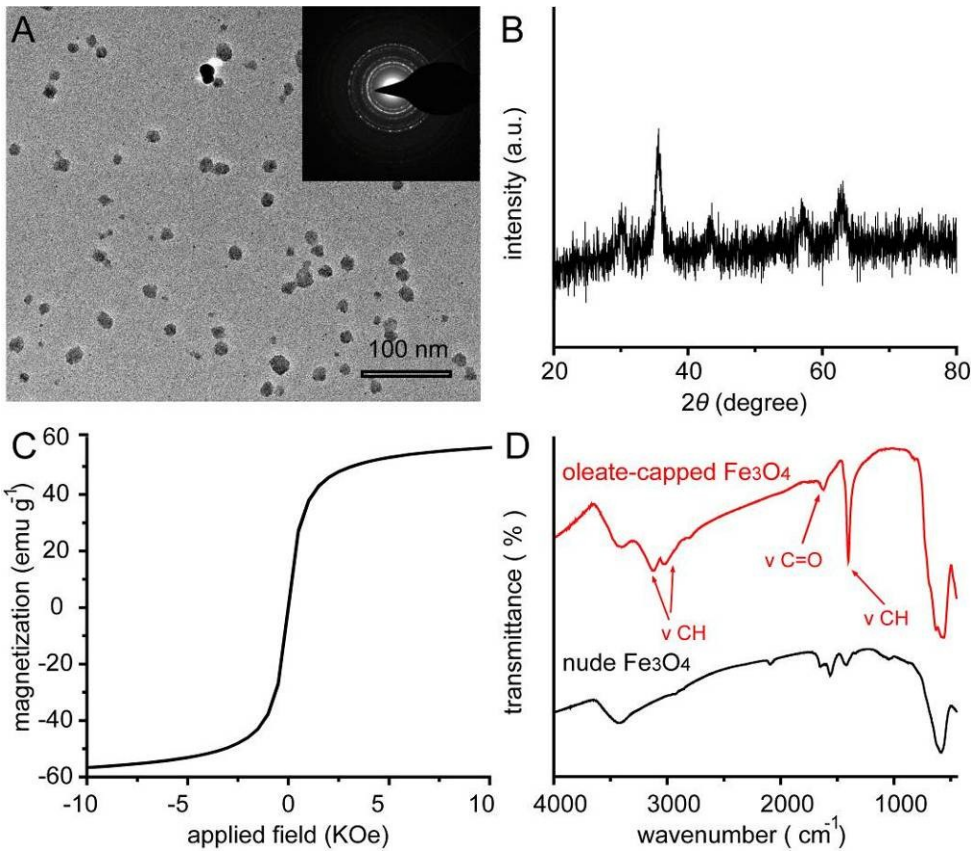

Figure S1. TEM image (A), wide-angle XRD pattern (B), room temperature magnetic hysteresis loops (C), and FT-IR spectra (D) of oleate-capped magnetic nanoparticles. Inset of A: SAED of oleate-capped magnetic nanoparticles.

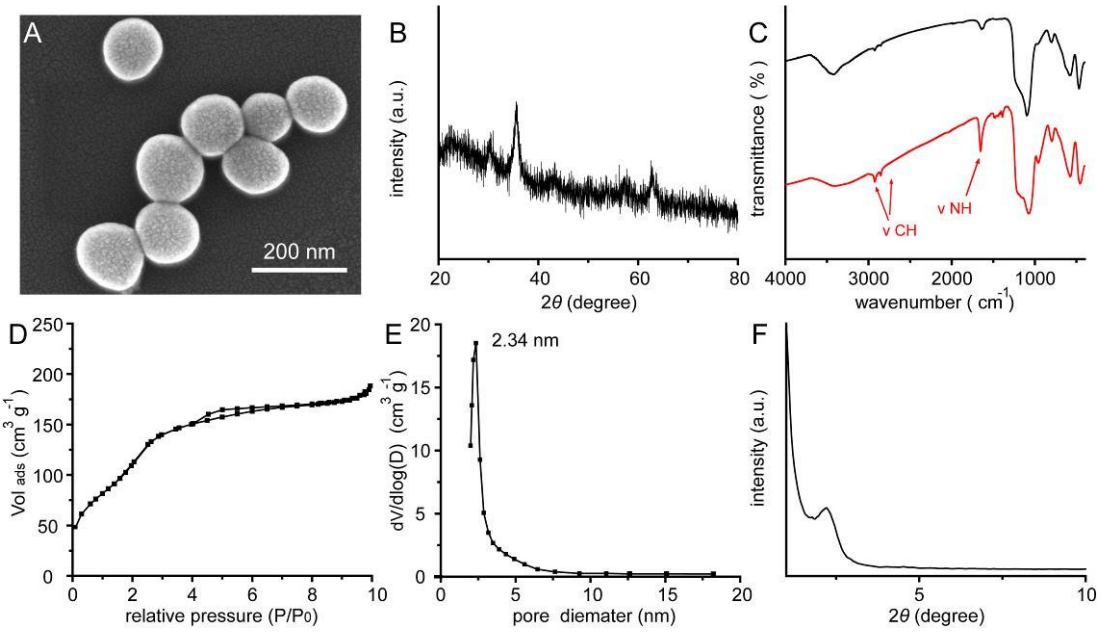

Figure S2. SEM image (A), wide-angle XRD pattern (B), FT-IR spectra (C), N_2 adsorption-desorption isotherm (D), related pore size distribution (E), as well as small-angle XRD pattern (F) of amido-modified magnetic nanovehicles. Black line in C: magnetic nanovehicles without any modification; Red line in C: amido-modified ones.

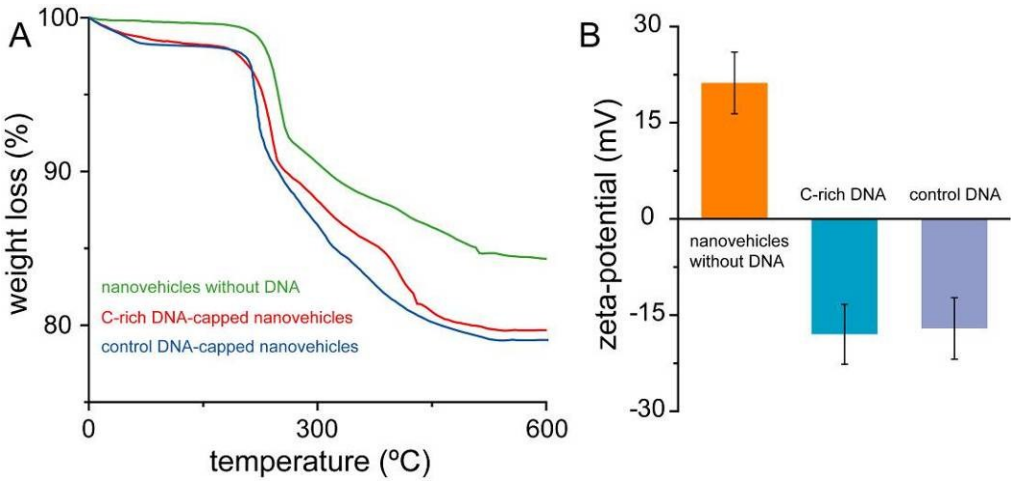

Figure S3. TGA curves (A) ζ potential (B) of C-rich DNA/control DNA-capped magnetic nanovehicles, as well as nude magnetic nanovehicles.

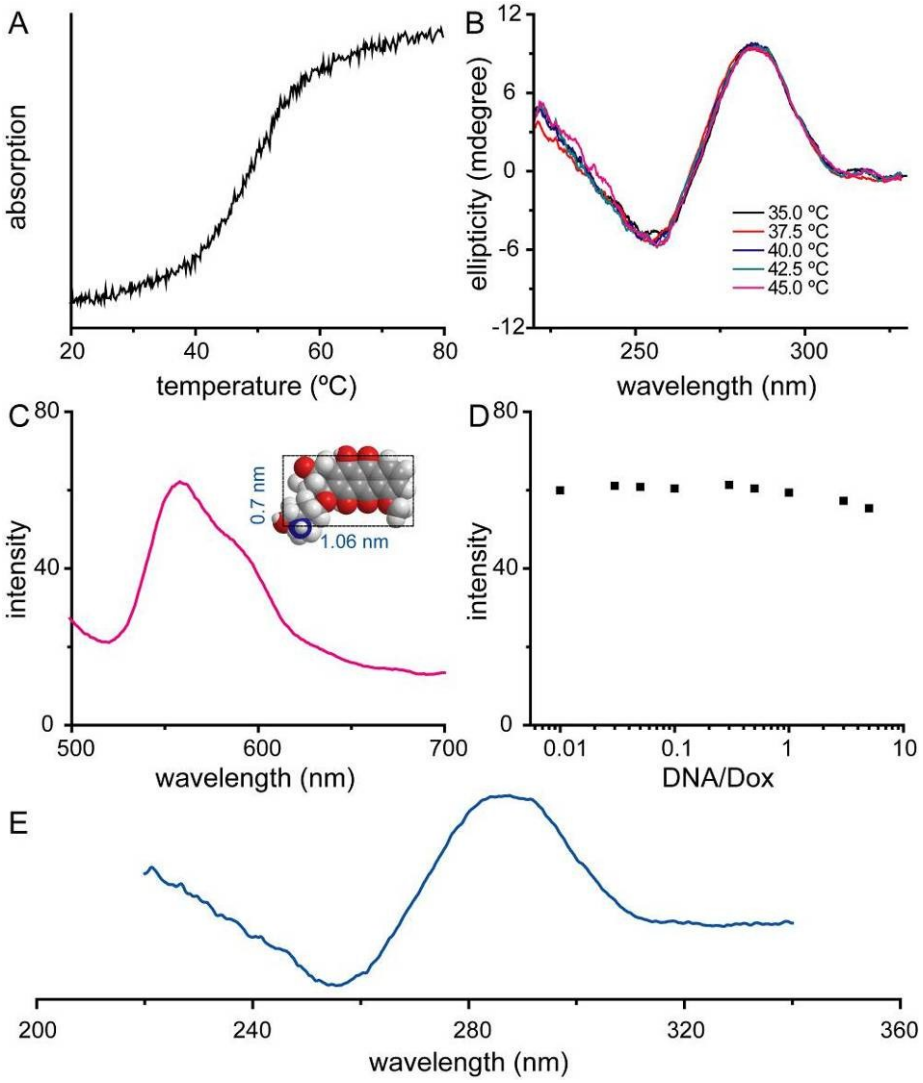

Figure S4. UV melting profile (A) and temperature-dependent CD spectra (B) of C-rich DNA in MES buffer (20mM, 100 mM NaCl, pH 5.0). Fluorescence emission spectra of Dox in acid MES buffer upon excitation at 480 nm (C). Inset: 3D chemical structure of Dox. Fluorescence intensity of various samples containing Dox and C-rich DNA with different mole ratio in acid MES buffer (D). CD spectra of supernatant liquid (20 mM MES, 100 mM NaCl, pH 5.0) containing C-rich DNA in the presence of magnetic nanovehicles (E).

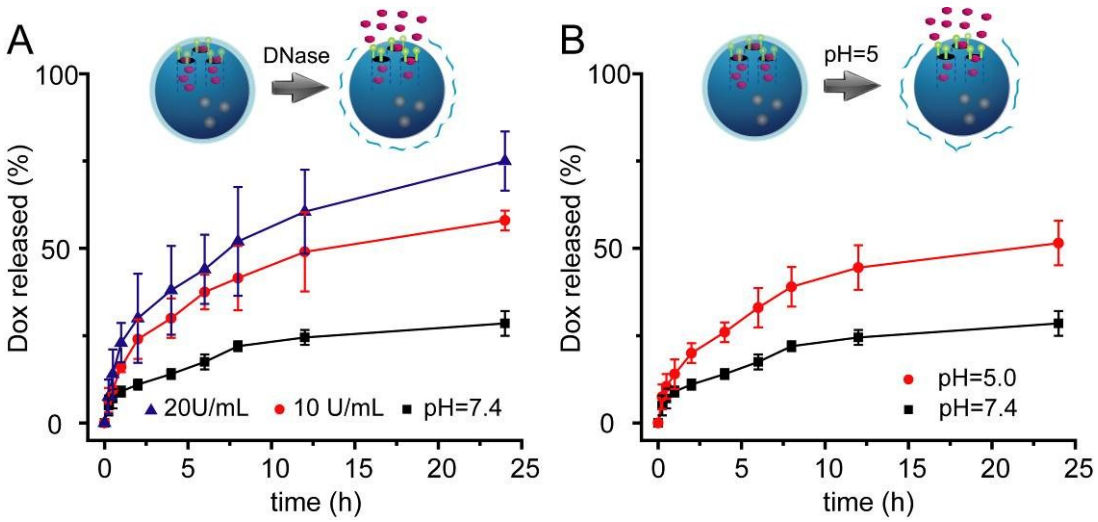

Figure S5. Release profiles of Dox from control DNA-capped magnetic nanovehicles upon different treatment: DNase with various concentrations (A) and different pH values (B).

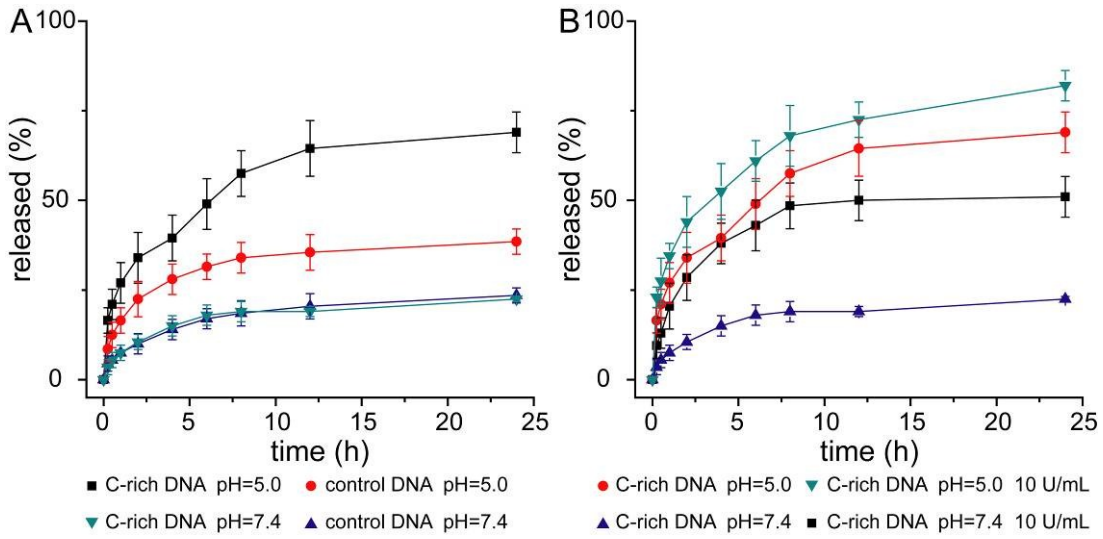

Figure S6. Release profiles of fluorescein sodium from C-rich/control DNA-capped magnetic nanovehicles upon different treatment: different pH values (A) and DNase (B).

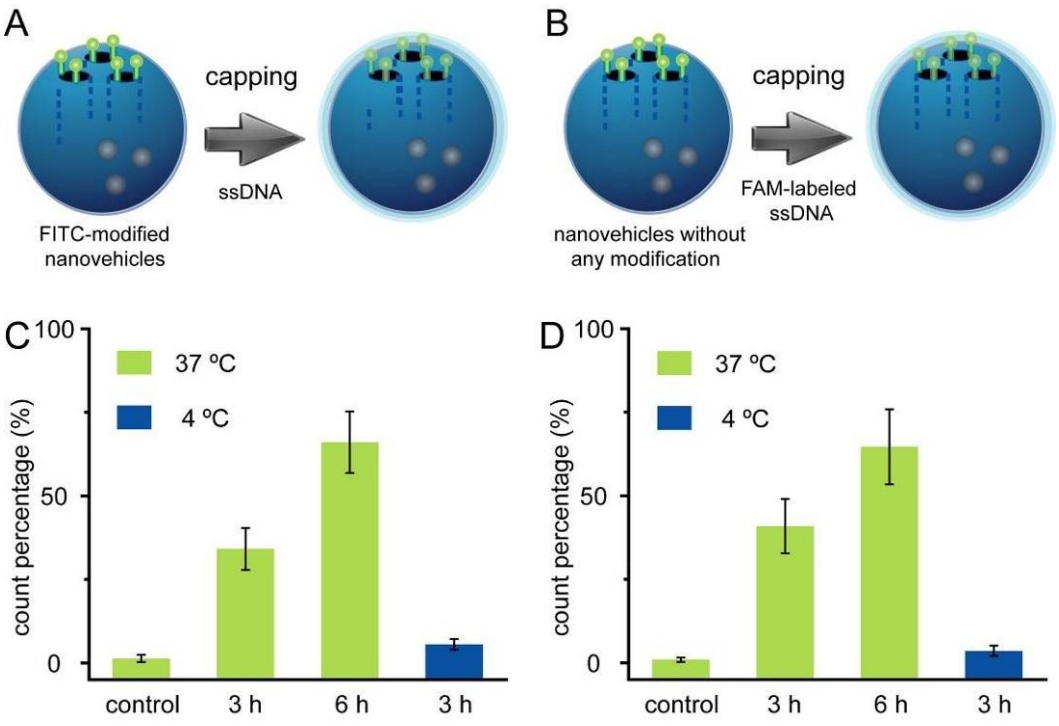

Figure S7. Schematic illustration of fluorescence DNA-capped nanovehicles: DNA-capped FITC-modified nanovehicles (A) and FAM-labeled DNA-capped nanovehicles (B). Time-dependent flow cytometry analysis to monitor the binding efficacy of above two nanovehicles towards Hela cells: DNA-capped FITC-modified nanovehicles (C) and FAM-labeled DNA-capped nanovehicles (D).

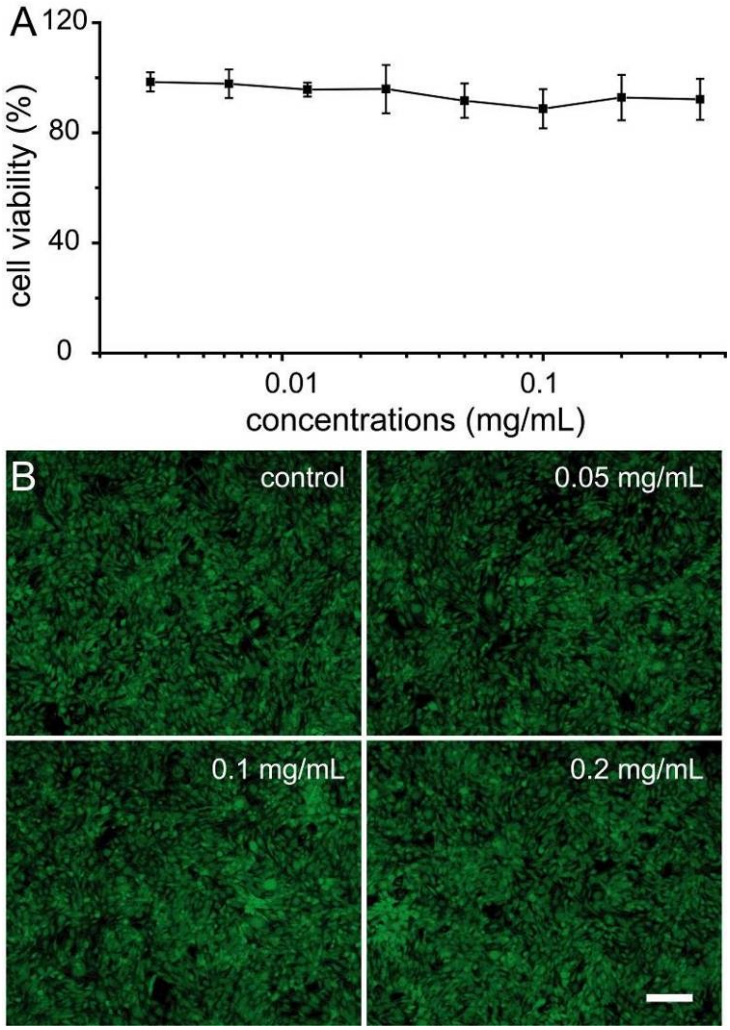

Figure S8. Viability (A) and live/dead stained images (B) of Hela cells incubated with C-rich DNA-capped magnetic nanovehicles. The scale bar is 500 μm.

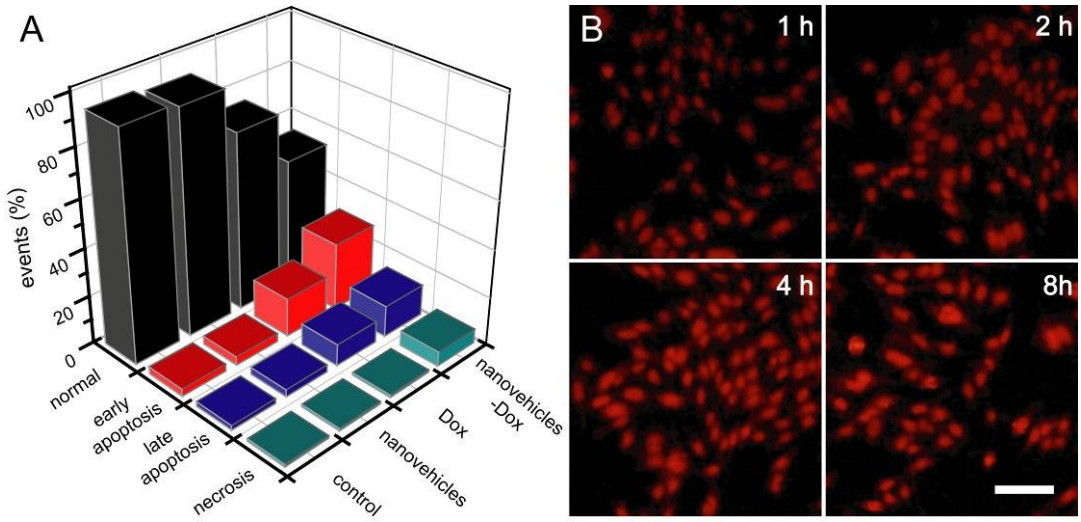

Figure S9. Apoptosis/necrosis analysis based on flow cytometry results of Hela cells upon different treatments (A). Time-dependent intracellular fluorescence imaging of drug after incubation with Dox-loaded C-rich DNA-capped magnetic nanovehicles (200 μg/mL) for different incubation periods (B). The scale bar is 100 μm.

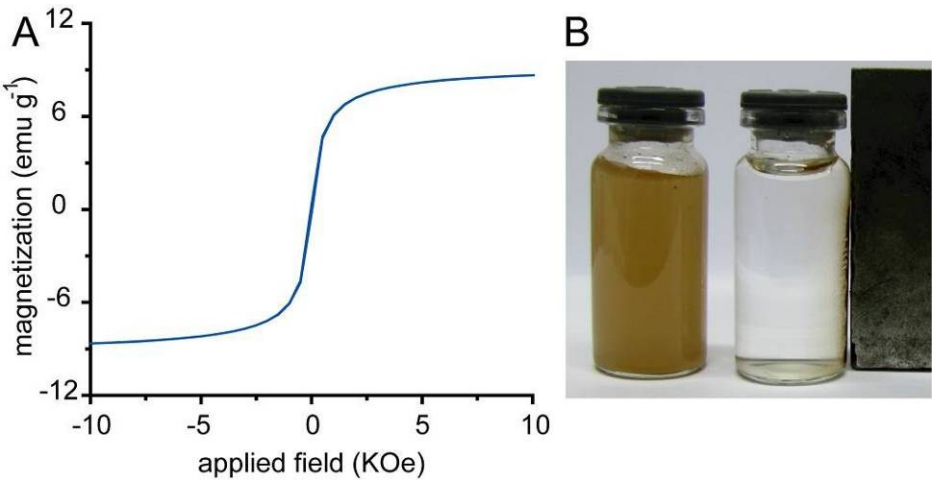

Figure S10. Room temperature magnetic hysteresis loops (A) and photo upon an assistant magnet (B) of C-rich DNA-capped magnetic nanovehicles.

Table S1. BET specific surface values, pore volumes, and pore sizes calculated from the N_2 adsorption-desporption isotherms of the as-prepared amido-decorated magnetic nanovehicles without C-rich DNA modification.

S_{BET} (m^2/g)	pore volume (cm^3/g)	pore size (nm)
474.1	0.388	2.34

Table S2. Comparison of C-rich DNA and control DNA as capping agents.

sample	ζ potential	DNA mass percentage	Dox loading amount
C-rich DNA	-18 mV	4.125 %	1.65 %
Control DNA	-17.1 mV	4.261 %	1.42 %