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Electronic Supplementary Information

pH-responsive spherical nucleic acid for intracellular lysosomes imaging and drug delivery system

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Experimental Methods

Preparation of Sterile, DNase/RNase-Free Solution. 0.2M Phosphate (NaH_2PO_4/Na_2HPO_4) buffer, 4M NaCl solution, 1XPBS buffer (137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, 4 mM Mg^{2+} pH = 8.0), Nanopure water (18.2 MΩ) and Au nanoparticles colloidal were treated by 1‰ diethylprocarbonate (DEPC; Sigma-Aldrich) at room temperature (RT) overnight, and then autoclaved. The tips and tubes also were sterile, and DNase/RNase-free.

Synthesis of pH-responsive SNA conjugates. The 13±1nm citrate-capped AuNPs were prepared as previously described (J. J. Storhoff, R. Elghanian, R. C. Mucic, C. A. Mirkin and R. L. Letsinger, J. Am. Chem. Soc., 1998, 120, 1959-1964). For the self-assembly scheme in PBS buffer, thiol-terminated capture DNA was reduced by excess tris (2-carboxyethyl) phosphine hydrochloride (TCEP) for one hour, and the purified via size-exclusion chromatography with a NAP5 Column (GE Healthcare). The thiol-terminated strands were mixed with Alexa-647 labeled reporter at 90 ˚C for 10 min, and then annealed to room temperature slowly. The DNA duplexes were added to gold nanoparticle colloids at a final concentration of 3.5 μM in AuNP colloids solution of 10nM, and the mixture was incubated overnight (~12 h). Then, the mixture was brought to 10 mM sodium phosphate (pH = 8.0) and 0.1% SDS and allowed to another 10 hours incubation. Subsequently, mixture was brought to 0.05M of NaCl by addition of 4M concentrated solution and this addition was repeated in 30 min intervals until a final concentration of 0.3 M NaCl. Then, the mixture was allowed to gently shake for 24h at room temperature. To purify the SNA conjugates, the mixture was centrifuged (15000 rpm, 30 min) and resuspended in 1XPBS (137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, 4 mM Mg^{2+} pH = 8.0) buffer three times. The concentration of SNA was determined by measuring the extinction at 520 nm ($\mathcal{E}=2.7\times10^{8}$ L mol^{-1} cm^{-1}). For Dox loading, Dox (25 μM) were added into SNA solution (70 nM). After 2h at room temperature, the mixture was purified by centrifugation for 3 times (13000 rpm, 25 min) to remove the unbinding Dox molecules. The resultant Dox-SNA solution was concentrated to ~75 nM and stored in 4 ˚C for future use.

Extracellular Fluorescence Measurements. The extracellular fluorescence measurements were performed on Jobin Yvon Fluorolog FL3-22. Alexa-647 and Dox are excited at 635 and 480 nm, and their emissions were recorded in ranges of 650-720 nm and 500-700 nm in 1 nm increments,

respectively. For the measurements shown in Fig. 1, 1.5 nM pH-responsive SNA is used. For the measurements of Dox release rate, 10 nM Dox-SNA conjugate is used. PH values are adjusted by alternately adding HCl or NaOH solution.

Cell Culture. HeLa cell line was obtained from Cell Bank of the Committee on Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China), and grown in Dulbecco's modified Eagles medium (DMEM, Hyclone) with 10% heat inactivated fetal bovine serum (FBS, Hyclone) and 1% Penicillin/Streptomycin (Gibco) in 5% CO_2 at 37 °C.

Cell Confocal Fluorescence Imaging. Cells were seed on coverglass bottom dish (diameter 35 mm, SPL Lifescience Co., Ltd.). For intracellular lysosome imaging, SNAs were added to media to a final concentration of 1.5 nM, and then removed at different time points of 3, 6, 9, 12h. After PBS buffer wash, cells were fixed by 4% paraformaldehyde and stained by 100 nM Lysotracker Green (Invitrogen) for 10 min at 37 °C in Opti-MEM. Then cells were washed again and observed under confocal fluorescence microscope with multiple excitations at wavelengths of 488 nm (lysotracker) and 633 nm (SNA). For Dox delivery, HeLa cells were incubated with Dox-SNA conjugates (6 nM) for 3 hours, then washed with PBS buffer and fixed by 4% HCHO for 10 min. Fluorescence images were obtained under Lecia SP5 confocal microscope with Ar laser 488.

Flow Cytometry Analysis. HeLa cells were treated with SNA alone (11 nM), control Dox-SNA conjugates (11 nM), pH-responsive Dox-SNA conjugates (11 nM) or free Dox (3.5 μM). Then cells were trypsinized, collected, and suspended in PBS buffer. Flow cytometry was performed with BD Analyses were performed on BD FACSCanto II system with BD FACSDiva software.

Cell Viability Assay. Cell viability assay was measured by using Cell Counting Kit (CCK-8). Cells were seeded in a 96-well plate at 3000 cells per well. Cells were treated with SNA alone (11 nM), control Dox-SNA conjugates (11 nM), pH-responsive Dox-SNA conjugates (11 nM) or free Dox (3.5 μM) for 2h, followed by PBS wash. After a final 24h incubation period, 10 μL CCK-8 solution was added to each well and incubated for 2 hours. The absorbance of each well was recorded at 450 nm.

Cell Apoptosis Measurement. HeLa cells were treated with SNA alone (11 nM), control Dox-SNA

conjugates (11 nM), pH-responsive Dox-SNA conjugates (11 nM) or free Dox (3.5 μM) for 2h, followed by PBS wash. After a final 24h incubation period, cells were trypsinized, collected, and suspended in PBS buffer and apoptosis was measured by treating cells with FITC-Annexin V and PI Cell Apoptosis Detection Kit (Invitrogen), according to the manufacturer's protocol. Analyses were performed on BD FACSCanto II system with BD FACSDiva software.

Oligonucleotide Sequences Used in This Study:

Table S1

Section	Name	Sequence (5'→3')
Lysosome imaging	pH-responsive DNA 66% CGC content	Thiolated end — TTTTTTTT GAGGAG GATTTGGCTAGACG CTCCTC TTTTT CTCCTC
	pH-responsive DNA-2 33% CGC content	Thiolated end — TTTTTTTT AAGGAA GATTTGGCTAGACG TTCCTT TTTTT TTCCTT
	Reporter	CGTCTAGCCAAATC—Alexa-647
	Control DNA	Thiolated end — TTTTTTTT GAGGAG GATTTGGCTAGACG CTCCTC TTTTT TCATAG
Dox delivery	pH-responsive DNA	Thiolated end — TTTTTTTT GAAGAG ACGACGACGACGACGAG CTTCTC TTTTT CTTCTC
	Dox loading	CTCGTCGTCGTCGTCGT
	Control DNA	Thiolated end — TTTTTTTT GAAGAG ACGACGACGACGACGAG CTTCTC TTTTT TCATAG

Dark yellow: Spacer; Blue: Double-stranded stem region; Green: Doubled-stranded loop region where reporter or Dox loads; Red: Overhanging tail.

Additional Data

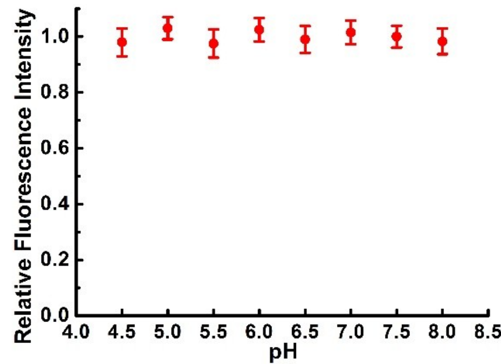

Fig. S1 The fluorescence intensity of Alexa-647 is insensitive to pH changes (50 nM reporter)

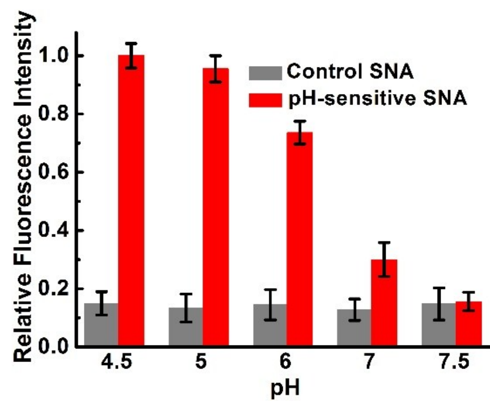

Fig. S2 When the sequence of overhanging region is random, the allosteric effect of DNA nanodevice cannot occur in acidic condition without Hoogsteen interactions. Hence, DNA strands remain in a linear conformation and the fluorescence intensity of SNA nanostructure is insensitive to pH changes.

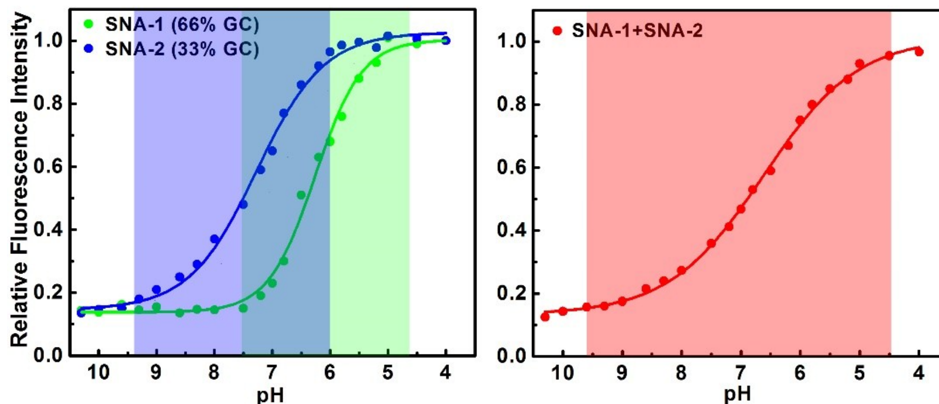

Fig. S3 The tunable pH window of DNA device can be extended by simply adjusting CGC content of triplex region. The SNA-1 (original version presented in manuscript and has a DNA triplex of 66% CGC) can respond to pH change within ~2.9 units (green window). While the SNA-2 with a triplet of 33% CGC content exhibits a working range of ~3.4 units (blue window). Strikingly, when two types of SNA are combined together (SNA-1: SNA-2=1:1), the tunable pH window is extremely extended to ~5.0 units (red window).

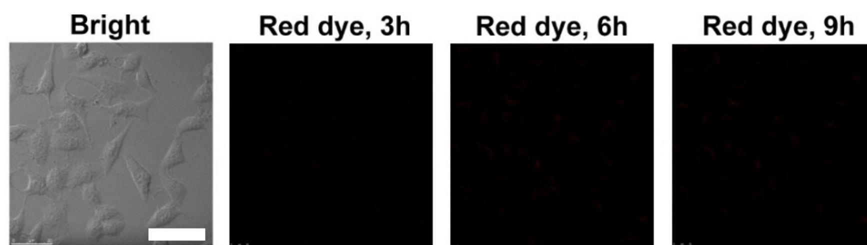

Fig. S4 As above mentioned, SNA conjugates with a random overhanging tail cannot sense pH changes and therefore cannot image intracellular lysosomes. Scale bar is 30 μm.

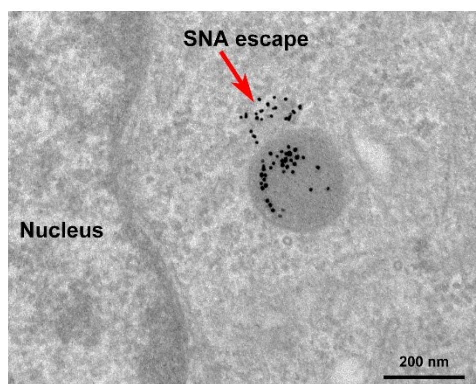

Fig. S5 Transmission electron microscopy (TEM) also demonstrates the cytosol and lysosomal distribution of SNA particles, suggesting the successful lysosomal escape.

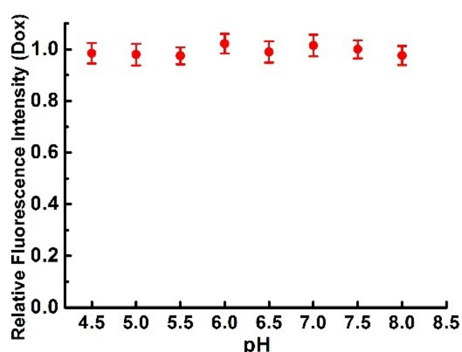

Fig. S6 The intrinsic fluorescence signal of Dox (3 μM) is independent on pH changes.

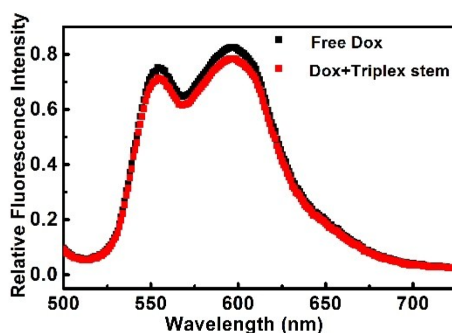

Fig. S7 In acidic environment, Dox molecules can be released from SNA nanocarriers. However, the triplex region contains GC/CG sequence, so Dox might reinsert into SNA carriers. We incubated Dox with SNA conjugates at acidic condition for 2 hours. There is no measurable difference in fluorescence intensity between free Dox and mixture. Hence, Dox homing is insignificant.

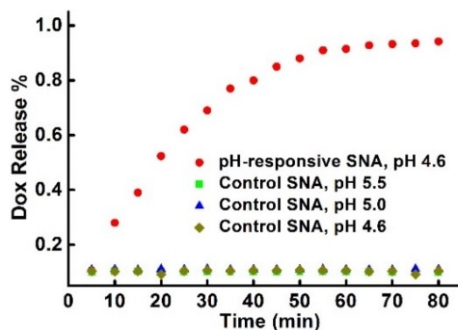

Fig. S8 The random overhanging tail cannot trigger the allosteric effect of DNA nanodevice. Thus, the control SNA nanocarriers cannot release Dox sufficiently in acidic environment.

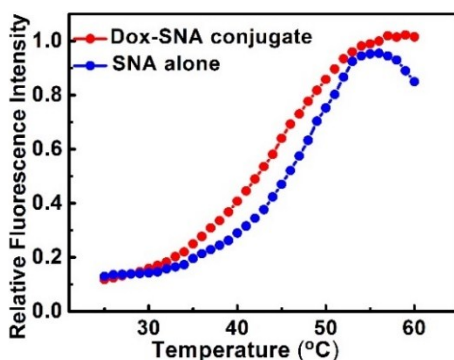

Fig. S9 The melting point of Dox-conjugated SNA (~59 ˚C) is higher than that of unloaded SNA (~55 ˚C), suggesting that Dox-SNA conjugate exhibits an enhanced thermostability.

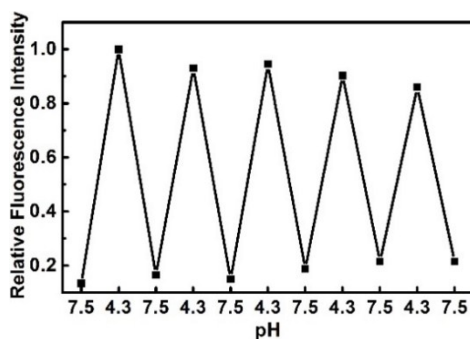

Fig. S10 The reversibility of Dox releasing and loading of SNA nanocarrier between pH 7.5 and 4.3.

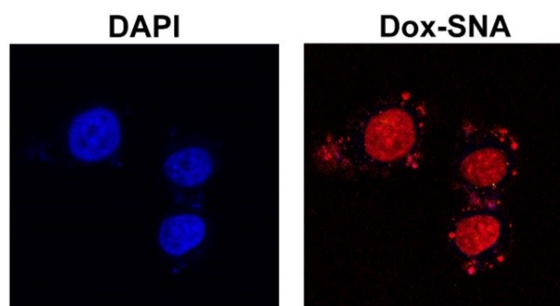

Fig. S11 The colocalization of nuclear dye and Dox fluorescence is consistent with Dox insertion into genomic DNA.

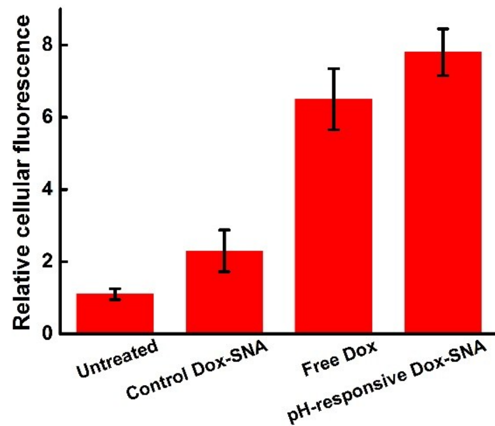

Fig. S12 The delivery capability evaluated by flow cytometry. The mean fluorescence intensity of pH-responsive SNA carrier is higher than that of free Dox.

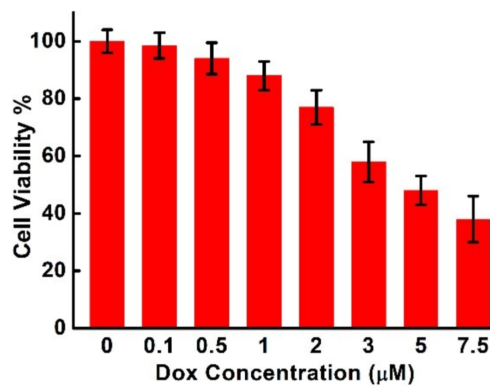

Fig. S13 The cell viability is measured under different Dox concentrations. The cell viability is significantly suppressed with the Dox concentration ≥ 3 μM.

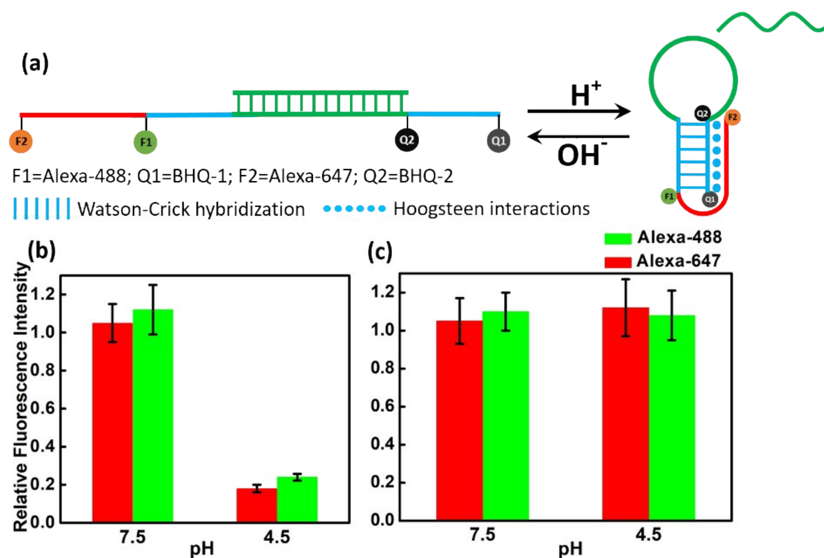

Fig. S14 (a) The DNA strand is labeled with fluorophores and quenchers as shown in the figure. In alkaline conditions, the DNA strand remains in a linear conformation and the fluorophores separate from the quenchers. Hence, the remarkable fluorescence emission can be detected. However, in the acidic conditions, the protonation of the overhanging tail (red) drives the DNA strand to switch into a loop-stem (green-blue) structure and the overhanging tail meanwhile binds to the stem region to

form a DNA triplet. On this occasion, the fluorophores are very close to the quenchers. As a result, the fluorescence signal will be quenched. (b) At the pH of 7.5, the fluorescence emissions from Alexa-488 and Alexa-647 are remarkable, indicating that the DNA strands adopt a linear conformation. At the pH of 4.5, the low fluorescence level of Alexa-488 suggests that DNA strands fold into a loop-stem architecture and the quenched Alexa-647 signal indicates that the overhanging tail binds to the stem to form a triplex structure. (c) When the sequence of overhanging tail is random, the allosteric effect of DNA strands cannot occur. The fluorescence signal therefore is insensitive to pH changes.

The sequence used in this section:

5'-(Alexa-647) CTCCTC TTTTT (Alexa-488) CTCCTC GATTTGGCTAGACG (BHQ-2) GAGGAG (BHQ1) -3'

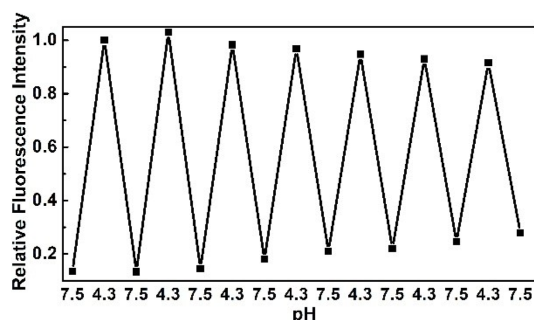

Fig. S15 The effective cycle number of the pH-responsive SNA in this study. If we define the ratio between maximal fluorescence intensity (F_{max}) and the minimum value (F_{min}) in each working cycle should be more than 3 ($F_{max} / F_{min} \geq 3$), the pH-responsive SNA in this study can be reused for 7 times.