Supporting Information

A Stable DNA Nanomachine Based on Duplex-triplex Transition for Ratiometric Imaging Instantaneous pH Changes in Living Cells

Mengqi Yang[a], Xiaoling Zhang[a*],Haipeng Liu[b], Huaizhi Kang[b], Zhi Zhu[b],Wen Yang[a*] ,Weihong Tan[b*]

a. Key Laboratory of Cluster Science of Ministry of Education, Beijing Key Laboratory of Photoelectronic/Electrophotonic Conversion Materials, School of Chemistry, Beijing Institute of Technology, 5 Zhongguancun Road, Beijing 100081, P. R. China.
E-mail: Zhangxl@bit.edu.cn. E-mail:wenyang@bit.edu.cn.
b. Center for Research at Bio/nano Interface, Department of Chemistry and Department of Physiology and Functional Genomics, Shands Cancer Center, UF Genetics Institute and McKnight Brain Institute, University of Florida, Gainesville, Florida 32611-7200, USA.
E-mali: tan@chem.ufl.edu.

ABSTRACT: DNA nanomachines are becoming useful tools for molecular recognition, imaging, diagnostics and have drawn gradual attention. Unfortunately, the present application of most DNA nanomachines are limited in vitro, so expanding their application in organism has becoming primary. Hence, a novel DNA nanomachine named t-switch, based on DNA duplex-triplex transition, are developed for motoring intracellular pH gradient. Our strategy is based on DNA triplex structure containing C^+-G-C triplets and pH-depended FRET. Our results indicate that t-switch is an efficient reporter of pH from pH 5.3 to 6 with a fast response of a few seconds. And the uptake of t-switch is speedy. In order to protects t-switch from enzymatic degradation, PEI is used for modification of our DNA nanomachine. At the same time, the dynamic range could be extended to pH 4.6-7.8. The successful application of this pH-depended DNA nanomachine and motoring spatiotemporal pH changes associated with endocytosis is a strong evidence of the possibility of self-assembly DNA nanomachine for imaging, targeted therapies and controllable drug delivery.

Design of t-switch and Fluorescence Property

Oligonucleotide Sequences used in this paper were shown in Table S1. Stock DNA solutions were prepared by dissolving lyophilized DNA in 20 mM PBS buffer and stored at -20□C until further use. 2 nmol of A, B and C were mixed in equimolar ratios in 20 mM PBS buffer. The resultant solution (1.6 μM) was heated to 90□C for 5 minutes, then cooled to the room temperature and equilibrated at 4□C overnight. The solution was diluted to 80 nM in appropriate buffer for fluorescence experiments unless otherwise mentioned.

Table S1. Oligonucleotide Sequences and modifications used in this paper.

Name	Sequence
Strand A	5'-Alex647-CCTTCCCTCTCTTTGGTTATAGGATCCTGCGGTCGG AGGCACCAGGCGTAAAATGTA-3'
Strand B	5'-Alex488-TTGGAAGGGAGAGATTGGTCTCTCCCTTCCTACAT TTTACGCCTGGTGCC-3'
Strand C	5'-CCGACCGCAGGATCCTATAA-3'

To confirm that fluorescent signal was due to the transition of conformation rather than fluorophore changes, we compared the emission spectrum of DNA nanomachine and solution which Alexa 488 was only contained at pH 5.0. The result (Figure S1a) shown that with the present of Alexa488 only, the peak at 520 nm did not drop and no new peak appeared at 675 nm. Therefore, fluorescent signal was produced by FRET which caused by the comformational change.

Temperature-depended fluorescence changes of t-switch were shown in Figure S1b. When temperature changed from 25°C to 35°C, there were no obvious changes. However, as temperature was increased successively, triplex structure was destroyed and fluorescent signal distinct changes were observed. The result indicated that t-switch could work normally at physiological temperature. The intensity of donor and acceptor fluorescence depended on

temperature was shown in Figure S1c.

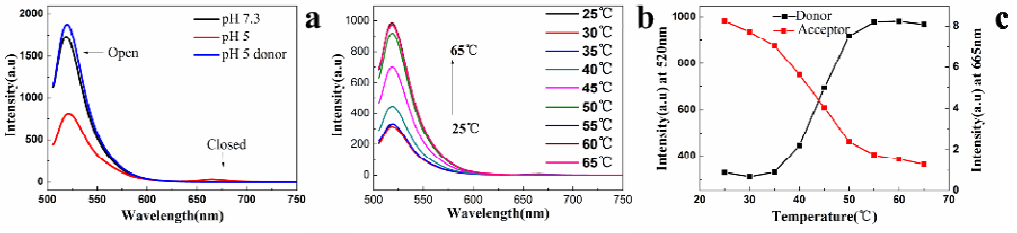

Figure S1. Optical spectrum to demonstrate open and closed conformations. a, DNA nanomachine labelled with Alexa 488 and Alexa 647 emission spectra of DNA nanomachine at pH 7.3 (black), pH 5.0 (red) and Alexa488 only (blue). b, Fluorescence melting experiments on DNA nanomachine at pH 5.0 in order to confirm its formation. c, Thermal denaturation profile of the DNA nanomachine. Donor intensity (black) at 520 nm and acceptor intensity (red) at 675 nm of the same as a function of temperature. Samples of 80 nM DNA nanomachine in 20 mM PBS, 100 mM NaCl at pH 5.0 were investigated.

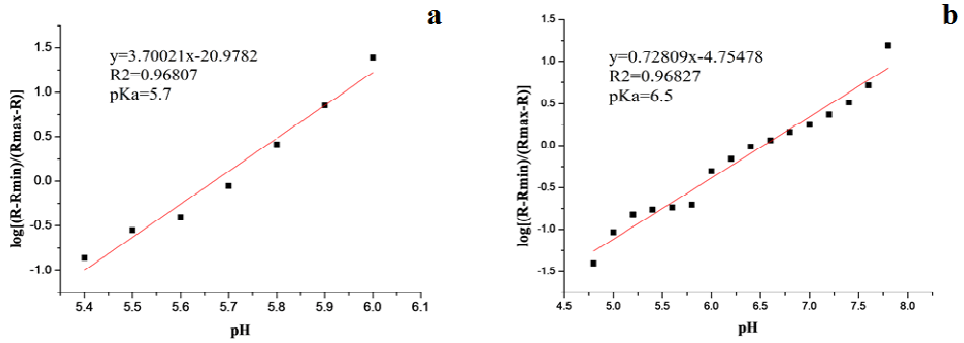

Figure S2. Calculation of the pKa of t-switch (a) and PEI complexed t-switch (b).

Design of PEI complexed t-switch and Fluorescence Property

1.6 μM of t-switch and PEI were mixed in a ratio of N/P=3. The mixture was set on vortex oscillation for 30 seconds, then wait for 30 minutes. In the following experiments, the mixture was diluted to 80 nM unless otherwise mentioned. This preparation of PEI/DNA complexes was shown in Figure S3a.

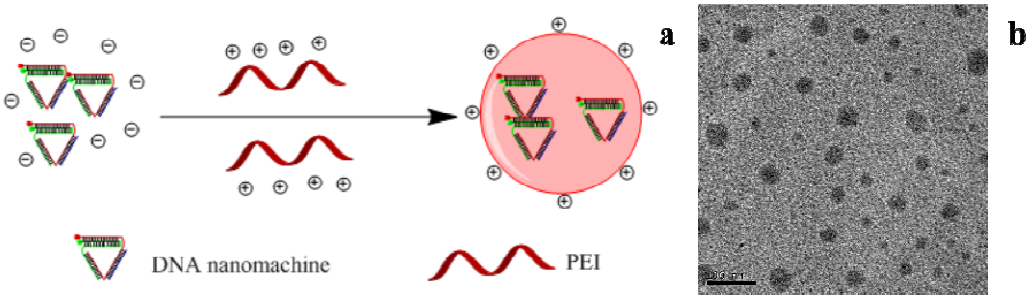

Figure S3. Preparation of PEI/DNA complexes. a, Principle of PEI/DNA complexes preparation. b, TEM image of PEI/DNA complexes (N/P=3).The scale bar is 100 nm.

In the experiment of stability of t-switch and PEI/DNA complexe, 2000 units/ml of DNase II was added in 80 nM of t-switch and PEI/DNA complexe respectively. The reaction temperature was kept constant at 36-37℃ and pH value was 5.0 in all the experiments. Fluorescence intensities were recorded at 675 nm with an excitation wavelength of 488 nm.

Spatiotemporal pH mapping in cytoplasm and endocytosis

RAW264.7 cells and HepG2 cells were incubated with 80 nM of t-switch and PEI/DNA complexe respectively in culture solution contained phenol red, then washed with PBS (20mM) for three times. The confocal fluorescence images were collected both in green channel (525 nm-575 nm) and red channel (650 nm-700 nm) with an excitation wavelength of 488 nm.

Lactate buffer pH 6.0: 107 mM NaCl, 4.2 mM KCl, 5 mM glucose, 10 mM HEPES, 10

mM MES, 1.1 mM CaCl2, 0.9 mM MgCl2, 20 mM Na-lactate.

After modification, surface of the PEI/DNA complexe was electropositive. Meanwhile the moieties of high amine density in PEI are important to overcome endosomal membrane barriers because their protonation potential contributes to endosome buffering as well as to membrane destabilization. Due to the two reasons above, PEI/DNA complexe was expected to enter living cells via nonspecific endocytosis. The possible pathways of t-switch and PEI/DNA complexe entering living cells and the working principle inside cells was shown in Figure S4. DNA nanomachines entered living cells through diffusion path and dispersed most in cytoplasm. They would remain an "open state" at neutral pH. If the pH value was reduced or t-switch entered some acid cell organelle, they might change into "closed state". In addition, PEI/DNA complexes were swallowed into endosome. It is indicated that pH value is 7.4-6.0 for the early endosome, 6.0-5.5 for the late endosome and 5.5-5.0 for the lysosome. The PEI/DNA complexes would change from "open state" to "closed state" with pH dropping.

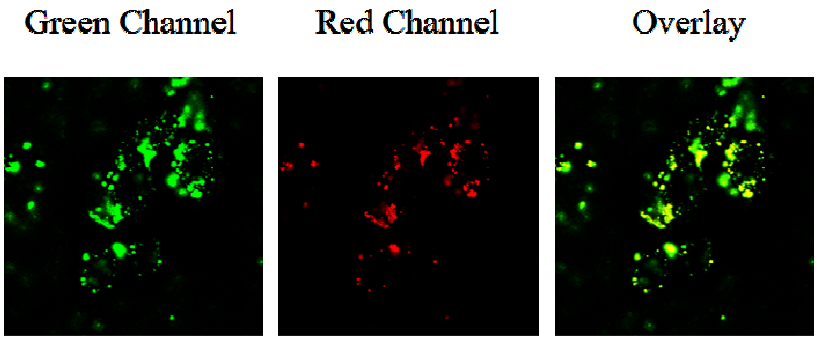

Figure S4. The cellular co-localization of t-switch (green channel) and Lyso-Tracker Red (red channel) after incubated with HepG2 cells for 15 minutes. It is demonstrated that t-switch locates both in cytoplasm and lysosome.

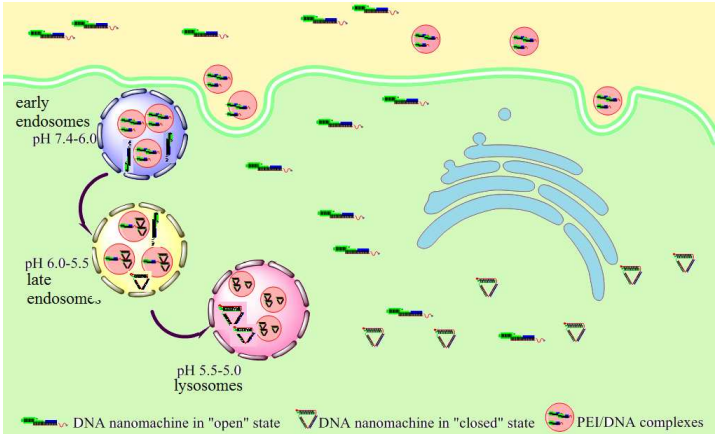

Figure S5. Pathways of t-switch and PEI/DNA complexe entering living cells and response mechanism.

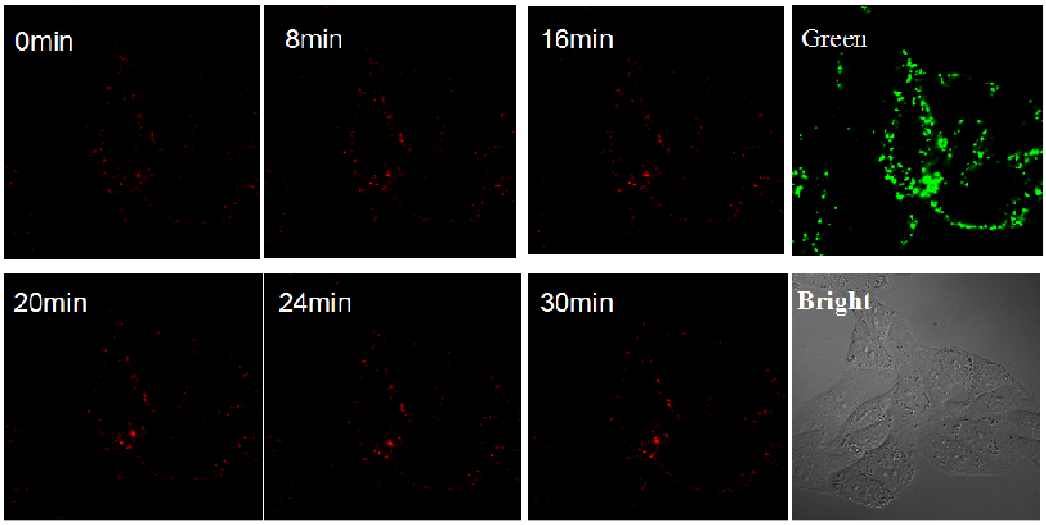

Figure S6. Time-depended confocal images of t-switch itself in HepG 2 cells.

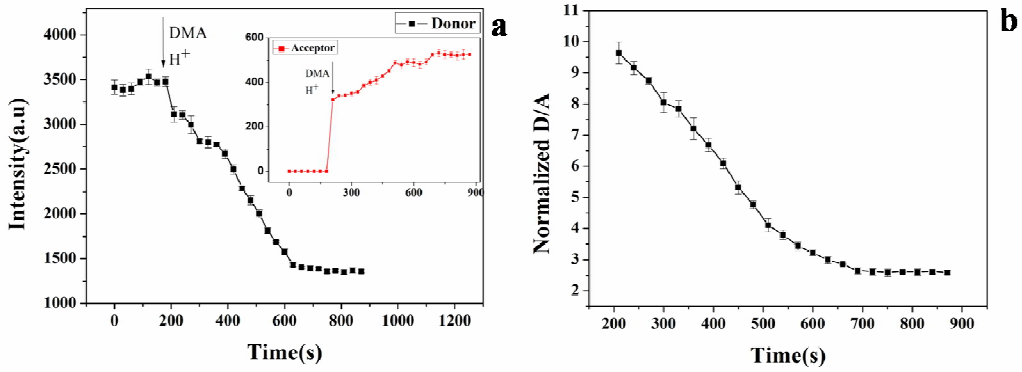

Figure S7. Intensity versus time traces for t-switch. a, The fluorescence intensity of t-switch in acidulated cells. Red curve is time-depended fluorescence intensity of Alexa-647 (acceptor) ,black curve is fluorescence intensity of Alexa-488 (donor). b, The normalized D/A intensity ratios in acidulated cells. Addition of DMA was shown by arrows

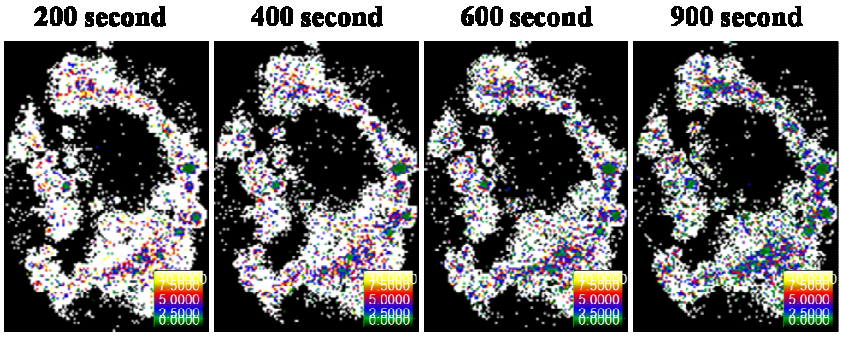

Figure S8. Partial enlarged ratiometric images of acidation HepG2 cells.

BCECF-AM are used to confirm this pH change caused by acidizing and the result are shown in Figure S10. HepG2 cells were incubated with 1 mM BCECF-AM for 30 minutes. The confocal fluorescence images were collected in green channel (510 nm-560 nm) with an excitation wavelength of 488 nm. According to the product information provided by suppliers, fluorescence intensity of BCECF-AM will weaken when pH value decreases. After acidified processing (b), fluorescent signal in green channel is decreased. So it shows that the pH is decreased after acidification treatment. The same result is also proved by other researchers[1-3].

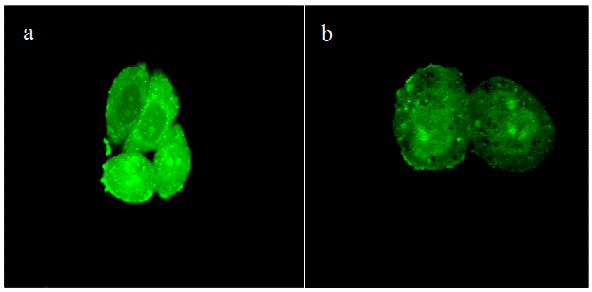

Figure S9. Confocal fluorescence images of HepG2 cells before (a) and after (b) acidizing. Significant fluorescence decrease can be observed. This means pH is reduced and the fluorescence changes of t-switch in Figure 2b is caused by pH changes.

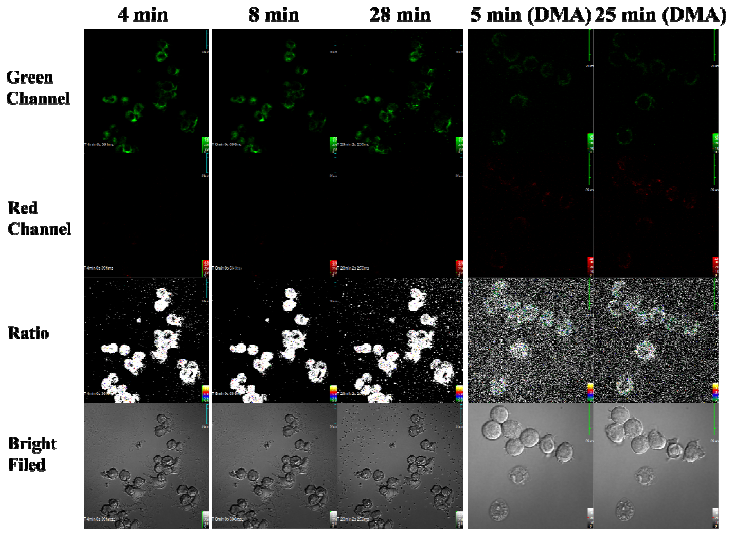

Figure S10. Confocal fluorescence images of t-switch in live RAW264.7 cells. Imaging of t-switch in nomal cells at 4min, 8min, 28min and after acidized are shown respectively.

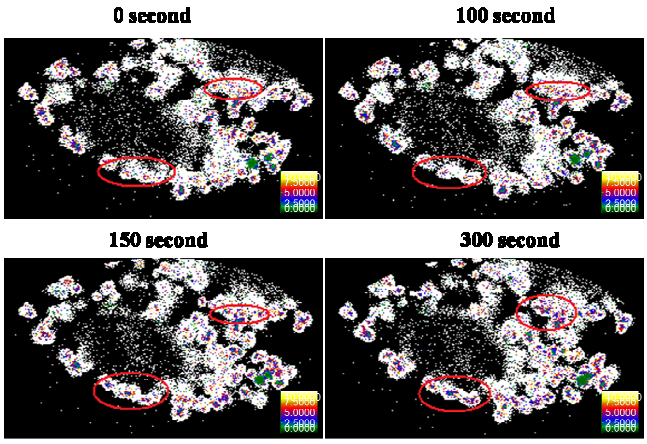

Figure S11. Partial enlarged ratiometric images of one HepG2 cell. Remarkable pH changes are observes such as area in the red circles.

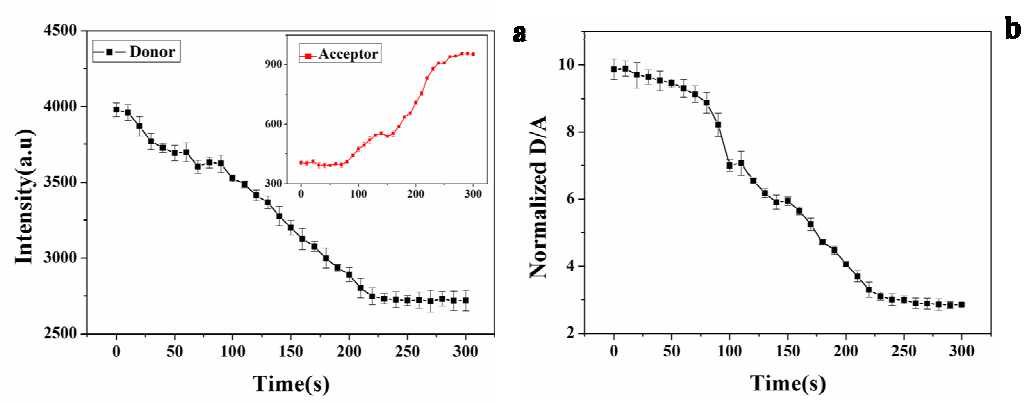

Figure S12. Intensity versus time traces for PEI/DNA complexes. a, Fluorescence intensity of PEI/DNA complexe in Hepg2 cells. Red curve is time-depended fluorescence intensity of Alexa-647 (acceptor) ,black curve is fluorescence intensity of Alexa-488 (donor).b, The normalized D/A intensity ratios of PEI/DNA complexe inside cells.

The cellular internalization of DNA nanomachine

To study the cellular internalization ability of t-switch, different kinds of cells were incubate with 150nM t-switch and dye-labeled single-stranded DNA(Strand A and Strand B). The results showed that after incubate with single-stranded DNA, minimal fluorescence were shown in Figure S12. Besides, another experiment was taken to study the entrance pathway of naked DNA nanomachine. In endocytosis inhibition experiment, cells were pretreated with a potassium-depleted (50 mM HEPES, 140 mM NaCl, 2.5 mM MgCl2, and 1 mM CaCl2) for 1 h at 37°C. Then t-switch and PEI/DNA complexe were added to cell-culture medium to obtain a final concentration of 150 nM. All the cells were incubated 10 minutes before flow cytometric analysis.

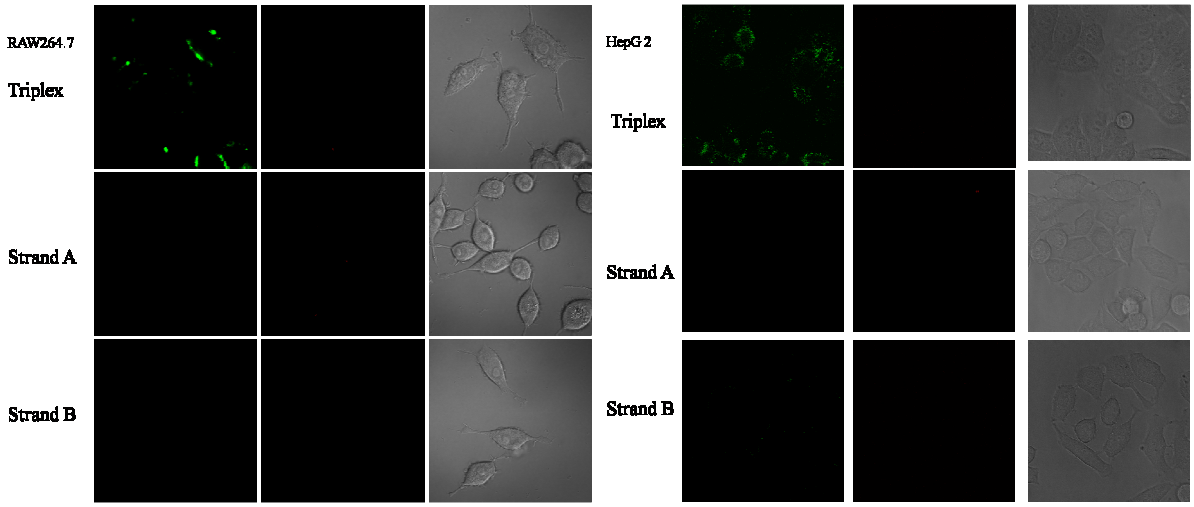

Figure S13. Confocal fluorescence images of HepG2 cells and RAW264.7 cells incubated with t-switch or single-stranded DNA for 15 minutes respectively.

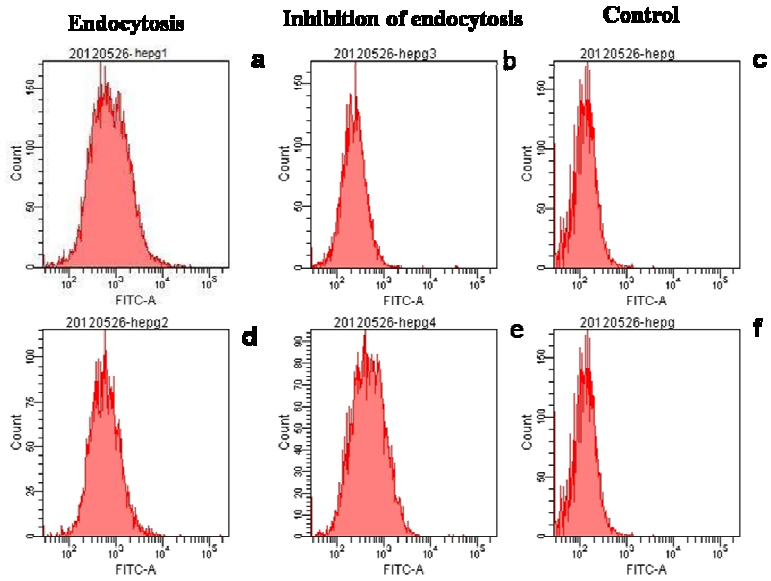

Figure S14. Flow cytometric analysis of the HepG2 cells incubated with PEI/DNA complexes (a,b) and t-switch (d,e) before or after inhibition of endocytosis. c and f are HepG2 cells without incubation. And the mean fluorescent intensity is shown in the histogram.

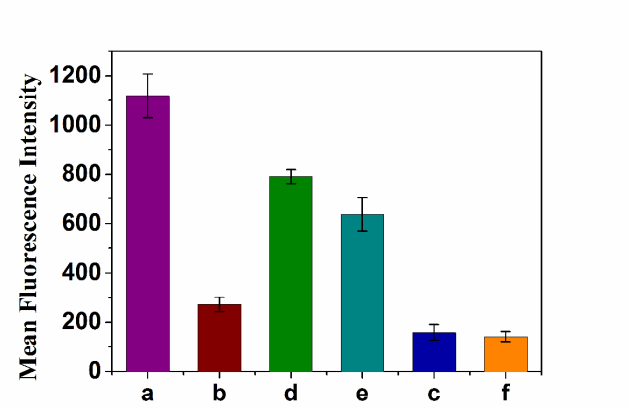

Figure S15. Mean fluorescent intensity of HepG2 cells in different conditions are shown in histogram. Concrete data is listed in Table S2.

Table S2. Mean fluorescent intensity of HepG2 cells in different conditions.

Tube Name	HepG2-1(a)	HepG2-3(b)	HepG2-2(d)	HepG2-4(e)	HepG2(c)	HepG2(f)
Mean FITC	1119	273	791	636	159	142
Events	4668	4933	4362	4890	4931	5005

References:

(1) Kaba, N. K.; Schultz, J.; Law, F. Y., et al. *Am. J. Physiol.* **2008**, 295, 1454-1463.
(2) Rich, IN.; Worthington-White, D.; Garden, OA.; Musk, P. *Blood.* **2000**, 95(4), 1427-34.
(3) Mirossay, L.; Mirossay, A.; Kocisová, E.; Radváková, I.; Miskovský, P.; Mojzis, J. *Physiol Res.* **1999**, 48(2), 135-41.