**Materials**

Oligonucleotides were purchased from Integrated DNA Technologies (Coralville, US), except for the Cy5 and BHQ-2 labeled H2 which were purchased from Sangon Biotech (Shanghai, China) Co., Ltd. 4,4'-dimethoxytrityl chloride was purchased from Acros Organics (Geel, Belgium). All other chemicals used in this work were purchased from Sigma-Aldrich without further treatment.

**DNA sequences used in this work**

1. Solution assay:

   H1: TAGGAGATCGGTGGTGCCTTGAGACCATGTGTAGATCTCAAGGCACCACCGATG GTTGTCAGAGCAC

   H2: CCTTGAGAT/BHQ-2/CTACACATGGTCTCAAGGCACCACCGCCATGTGTAGA/Cy5/

   Standard Initiator DNA: TCTCAAGGCACCACCGATCTCCTA

   Control H1: GTACTGATCGGTGGTGCCTTGAGACAAAGTTCTCAAGGCACCACCGAT

1.1 NaA43 DNAzyme:

   Substrate strand: TCTCAAGGCACCACCGATCTCCTATrAGGAAGTTCCGCCGCCGC

   Enzyme strand: GCGGCGGCGGAACCAGGTCAAAGGTGGGTGAGGGGACGCCAAGAGTC CCCGCGGTTAGGAGATCGGT

1.2 Non-cleavable NaA43 DNAzyme:

   Non-cleavable substrate strand: TCTCAAGGCACCACCGATCTCCTATAGGAAGTTCCGCCGCCGC

   Enzyme strand: GCGGCGGCGGAACCAGGTCAAAGGTGGGTGAGGGGACGCCAAGAGTCCCCG CGGTTAGGAGATCGGT

   Non-cleavable NaA43 DNAzyme is in which the rA base at the cleavage site of the substrate strand is replaced with a deoxyribose base.

1.3 Selectivity assay of CHA

   One base mismatch substrate strand: TCTCAAGGCACCACCGATCTTCTATrAGGAAGTT CCGCCGCCGC

   Two bases mismatch substrate strand: TCTCAAAGCACCAACGATCTTCTATrAGGAAGTT CCGCCGCCGC

   Mismatch enzyme strand: GCGGCGGCGGAACCAGGTCAAAGGTGGGTGAGGGGACGC CAAGAGTCCCCGCGGTTAGAAGATCGGT

2. Intracellular study

H1: TAGGAGATCGGTGGTGCCTTGAGACCATGTGTAGATCTCAAGGCACCACCGATG

GTTGTCAGAGCAC

H2: CCTTGAGAT/BHQ-2/CTACACATGGTCTCAAGGCACCACCGCCATGTGTAGA/Cy5/

H2 for localization study: CCTTGAGATCTACACATGGTCTCAAGGCACCACCGCCATGTGTAGA/Cy5/

2.1 Active caged NaA43 DNAzyme

Active caged substrate strand: TCTCAAGGCACCACCGATCTCCTAT /iNiBenz-rA/ GGAAGTTCCG

CCGCCGC

Enzyme strand: GCGGCGGCGGAACCAGGTCAAAGGTGGGTGAGGGGACGCCAAGAGTCCCCGCG

GTTAGGAGATCGGT

2.2 Non-cleavable NaA43 DNAzyme

Non-cleavable substrate strand: TCTCAAGGCACCACCGATCTCCTATAGGAAGTTCCGCCGCCGC

Enzyme strand: GCGGCGGCGGAACCAGGTCAAAGGTGGGTGAGGGGACGCCAAGAGTCCCCGCGGTTAGGA

GATCGGT

Non-cleavable NaA43 DNAzyme is which the rA base at the cleavage site of the substrate strand is replaced with a deoxyribose base.

2.3 Inactive caged NaA43 DNAzyme

Active caged substrate strand: TCTCAAGGCACCACCGATCTCCTAT /iNiBenz-rA/ GGAAGTTCCGCC

GCCGC

Inactive enzyme strand:

GCGGCGGCGGAACCAGGACAAAGGTGGGTGAGGGGACGCCAAGAGTCCCCGCGGTTAGGAG

ATCGGT

Inactive caged NaA43 DNAzyme is which a conserved base in the enzyme strand that play a key role in the activity is replaced with another base.

**Synthesis of photocaged adenosine phosphoramidite**

Photocaged adenosine phosphoramidite were synthesized following a reported method.[S1] Mass spectra of the photocaged adenosine phosphoramidite were obtained from Waters Quattro II spectrometer in positive-ion mode and collected from 500-1200 m/z.

**Fluorescence assay**

Substrate strands and enzyme strands (1:1.1 ratio) were added to the reaction buffer (10 mM PB, 140 mM KCl, pH 7.4), heated to 80 ℃ for 3 min, then cooled to room temperature slowly to form DNAzyme structure. H1 and H2 were added to reaction buffer separately, followed by heating to 95 ℃ for 5 min, then put on ice for 5 min, and finally

transfered to room temperature for 1 h before further use. Fluorescence data was obtained by a HORIBA Jobin Yvon FluoroMAX-P using 635 nm excitation and 661 nm emission.

For sensitivity assay experiments, buffer K (10 mM PB, 140 mM KCl, pH 7.4) and buffer Na (10 mM PB, 140 mM NaCl, pH 7.4) were prepared first. By mixing these two buffers with different ratios, reaction buffers with different concentrations of $Na^+$ were obtained. 100 nM DNAzyme, 100 nM H1, and 150 nM H2 were added to the reaction buffer, incubated at 37 ℃ for 3h, and the fluorescence spectra was taken.

For selectivity assay experiments, 100 nM DNAzyme, 100 nM H1, and 150 nM H2 were added to Bis-Tris buffer (20 mM Bis-Tris, 140 mM KCl, pH 7.4), then different metal ions were added into the reaction solution. Samples were incubated at 37 ℃ for 3 h, and the fluorescence spectra was taken.

**Real-time fluorescence assay**

The reaction buffers with different concentration of sodium ions were prepared by mixing buffer Na and buffer K with various ratios. In the reaction buffers containing different concentration of sodium ions, 100 nM DNAzyme, 100 nM H1, and 150 nM H2 were added. Real-time fluorescence spectra were obtained using TECAN Infinite M1000 PRO. The excitation is 635 nm and emission is 661 nm. The reactions were performed at 37 ℃ and the data were collected every 30 s.

**Gel electrophoresis assay**

Gel electrophoresis analysis was performed using 12% (w/w) native polyacrylamide gel electrophoresis (PAGE) gels in 1 × TBE buffer. The electrophoresis was then performed at a constant potential of 250 V for 25 min with a load of 10 mL of sample in each lane at room temperature. After electrophoresis, the gel was stained by ethidium bromide and imaged via a Bio-Rad Gel Doc 2000 imaging system.

**Cell culture and DNA transfection**

HeLa cells were cultured in Dulbecco's modification of Eagle's medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), 100 U/mL penicillin, and 100 mg/mL streptomycin, and were incubated at 37℃ in a humidified 5% $CO_2$ incubator. Before imaging, cells were plated in 35 mm glass bottomed culture dishes (MatTek Corp., Ashland, MA) and grown for 48 h to reach 80-90% confluency.

DNA transfection were performed using G8 cationic polypeptides based on the methods in a previous paper.[S2] Briefly, 100 nM DNAzyme, 100 nM H1, and 150 nM H2 were mixed with 15 μL G8 polypeptide (0.2 mg/ml, pH 6.0) for 25 min at room temperature to form the G8-DNA complex, then this G8-DNA complex was mixed in Opti-MEM, followed by adding this solution to cells grown in a plate and incubated for 4 h.

**Confocal microscopy imaging**

For co-localization studies, HeLa cells were transfected using G8 polypeptides for 4 h, then washed with PBS, followed by staining with Hoechst 33258 for 20 mins in normal cell culture medium, then washed with PBS again, and staining with either LysoTracker Red DND-99 or ER tracker Red for 20 mins. Images were obtained using a Zeiss LSM 880 high resolution confocal microscope at 63× magnification. Fluorescence of Hoechst 33258 was obtained by excitation at 405 nm and collected over 420-520 nm; Fluorescence of ER Tracker was obtained by excitation at 561 nm and measured over 575-630; Fluorescence of LysoTracker was obtained by excitation at 561 nm and measured over 575-630.

For endogenous $Na^+$ detection, after transfection for 4 h, HeLa cells were washed with PBS to remove the untransfected G8-DNA complex, then immersed in Opti-MEM and irradiated with UV light at 365 nm for 30 min. Cells were then washed with PBS and taken for imaging immediately, or incubated with normal cell culture medium for a further 3h before imaging. Confocal microscopy images were collected using a Zeiss LSM 880 high resolution confocal microscope at 63× magnification. Fluorescence emission was from 650-790 nm, with excitation at 635 nm.

For detection of varying $Na^+$ concentrations, HeLa cells were transfected with G8 cationic polypeptide for 4 h, washed with PBS, then immersed in Opti-MEM and irradiated with UV light at 365 nm for 30 min. After washing with PBS, cells were immersed in influx buffer solution containing various $Na^+$ concentration which were prepared by mixing two influx buffers with different ratios. (buffer A: 10 mM HEPES, 30 mM NaCl, 120 mM $Na^+$ gluconate, 2 mM $MgCl_2$, 2 mM $CaCl_2$, pH 7.4; buffer B: 10 mM HEPES, 30 mM KCl, 120 mM $K^+$ gluconate, 2 mM $MgCl_2$, 2 mM $CaCl_2$, pH 7.4). Then, gramicidin D, monensin and ouabain were added to reach final concentrations of 3 μM, 10 μM and 100 μM, respectively. After influx of $Na^+$ for 25 min, cells were washed with PBS and incubated with normal cell culture medium for 1 h. Then cells were taken for confocal imaging using a Zeiss LSM 880 high resolution confocal microscope at 63× magnification.

**Flow cytometry assay**

For endogenous $Na^+$ detection, HeLa cells were plated in a 24-well plate and grown for 48 h to reach 80-90% confluency. After transfection with G8 cationic polypeptide for 4 h, cells were washed with PBS, immersed in Opti-MEM and irradiated with UV light at 365 nm for 30 min, then cells were either washed with PBS and taken for assay immediately or incubated with normal cell culture medium for further 3 h. Then cells were washed with PBS and detached from the 24-well plate by 0.05% trypsin. The suspended cells were centrifuged and dispersed in PBS for flow cytometry. Flow cytometry was obtained using a BD FACS Canto system. Totally 10000 cells were counted for each sample.

For detection of varying $Na^+$ concentrations, HeLa cells were plated in a 24-well plate for 48 h to reach 80-90% confluency, and then transfected with G8 cationic polypeptide for 4 h, washed with PBS, finally immersed in Opti-MEM

and irradiated with UV light at 365 nm for 30 min. After washing with PBS, cells were immersed in the influx buffer solutions containing various $Na^+$ concentrations which were prepared by mixing two influx buffers (buffer A and buffer B) with different ratios. Then, gramicidin D, monensin and ouabain were added to reach final concentrations of 3 μM, 10 μM and 100 μM, respectively. After influx of $Na^+$ for 25 min, cells were washed with PBS and incubated with normal cell culture medium for 1 h. Then cells were washed with PBS and detached from the 24-well plate by 0.05% trypsin. The suspended cells were centrifuged and dispersed in PBS for flow cytometry. Flow cytometry was obtained using a BD FACS Canto system. Totally 10000 cells were counted for each sample.

**Reference**

(S1) Chaulk, S. G.; MacMillan, A. M. Nat. Protoc. 2007, 2, 1052-1058.

(S2) Torabi, S.-F.; Wu, P.; McGhee, C. E.; Chen, L.; Hwang, K.; Zheng, N.; Cheng, J.; Lu, Y. *Proc. Natl. Acad. Sci.* **2015**, *112*, 5903-5908.

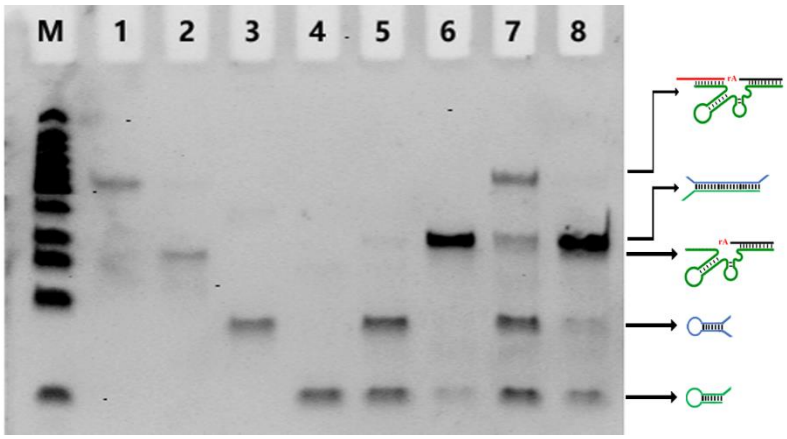


**Figure S1.** 12% native PAGE gel for DzCHA amplification reaction. Lane M: DNA marker; Lane 1: NaA43 DNAzyme in buffer without $Na^+$; Lane 2: NaA43 DNAzyme in buffer with $Na^+$ (100 mM); Lane 3: H1; Lane 4: H2; Lane 5: H1+ H2; Lane 6: Standard initiator + H1 + H2; Lane7: NaA43 DNAzyme + H1 + H2 in buffer without $Na^+$; Lane 8: NaA43 DNAzyme + H1 + H2 in buffer with $Na^+$ (100 mM).

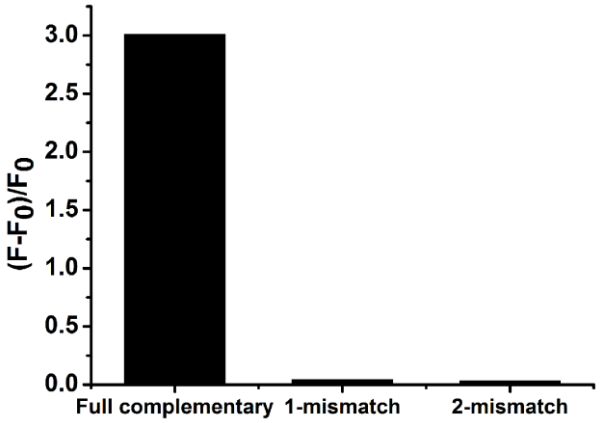


**Figure S2.** Selectivity of CHA reaction to full complementary, one base mismatch and two bases mismatch of the released initiator DNA strand from DNAzymes cleavage reaction.

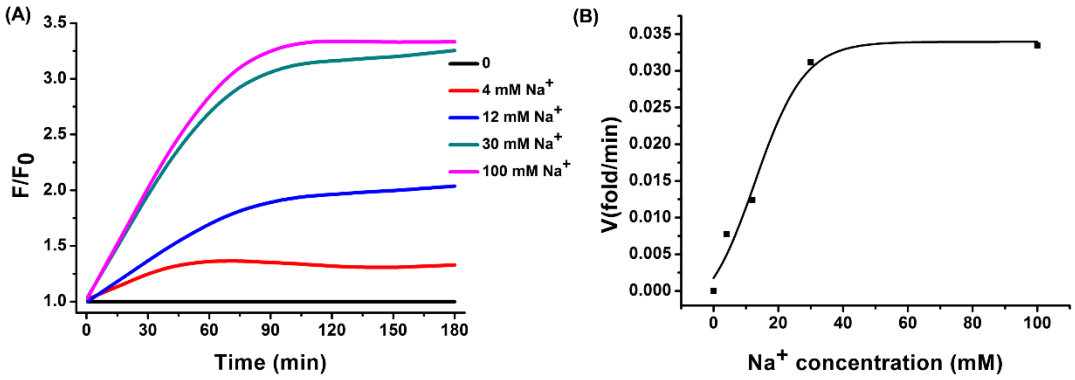


**Figure S3.** (A) Real-time fluorescence responses of DzCHA probe over time. From bottom up, the concentrations of sodium ions were 0, 4 mM, 12 mM, 30 mM and 100 mM, respectively. (B) Plot of initial fluorescence increase rates versus the concentrations of sodium ions. The initial fluorescence increase rates were calculated using the real-time fluorescence curves by fitting the data from 0-5 min.

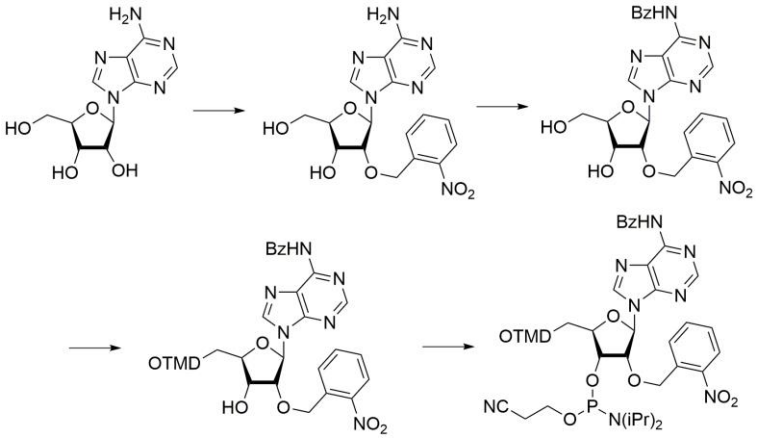


**Figure S4.** Synthesis of photocaged adenosine phosphoramidite.

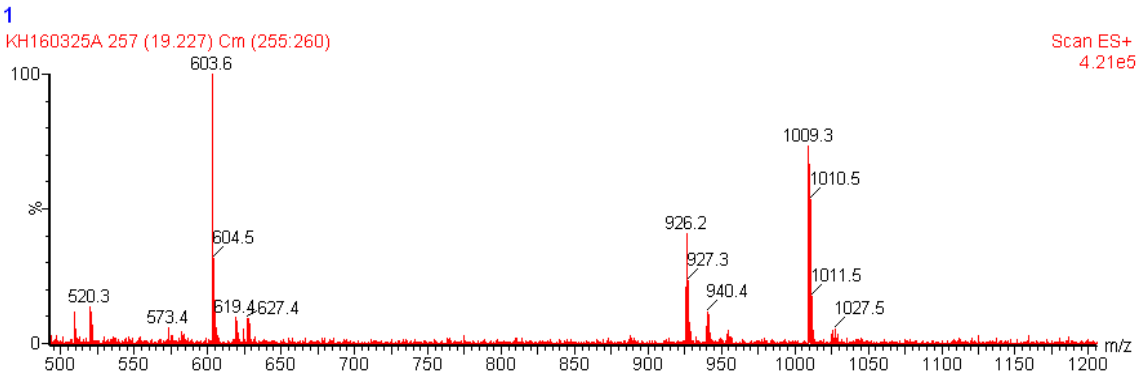


**Figure S5.** Mass spectrum of photocaged adenosine phosphoramidite. Expected mass: 1009.4013.

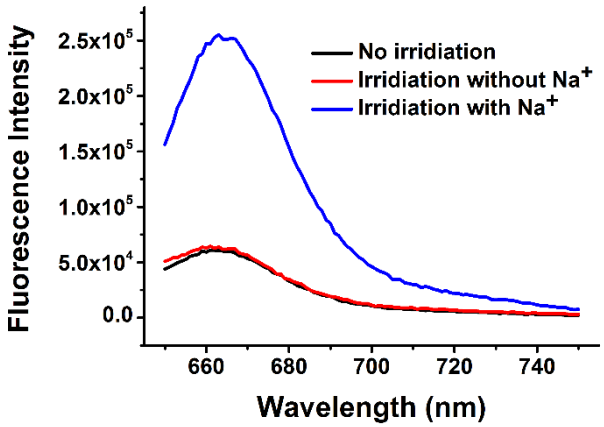


**Figure S6**. Fluorescence spectra for the photocaged DzCHA probe. Black line: caged NaA43 DzCHA in buffer with $Na^+$ (100 mM) without irradiation. Red line: caged NaA43 DzCHA in buffer without $Na^+$ with irradiation at 365 nm for 30 min. Blue line: caged NaA43 DzCHA in buffer with $Na^+$ (100 mM) with irradiation at 365 nm for 30 min.

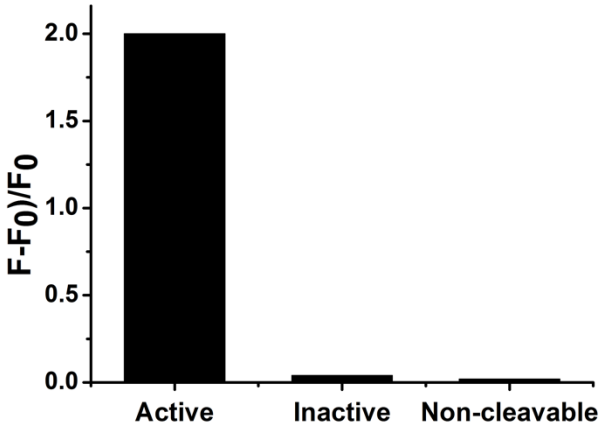


**Figure S7**. Performance of the DzCHA probe in HeLa cell lysate, and comparison with inactive DzCHA and non-cleavable DzCHA.

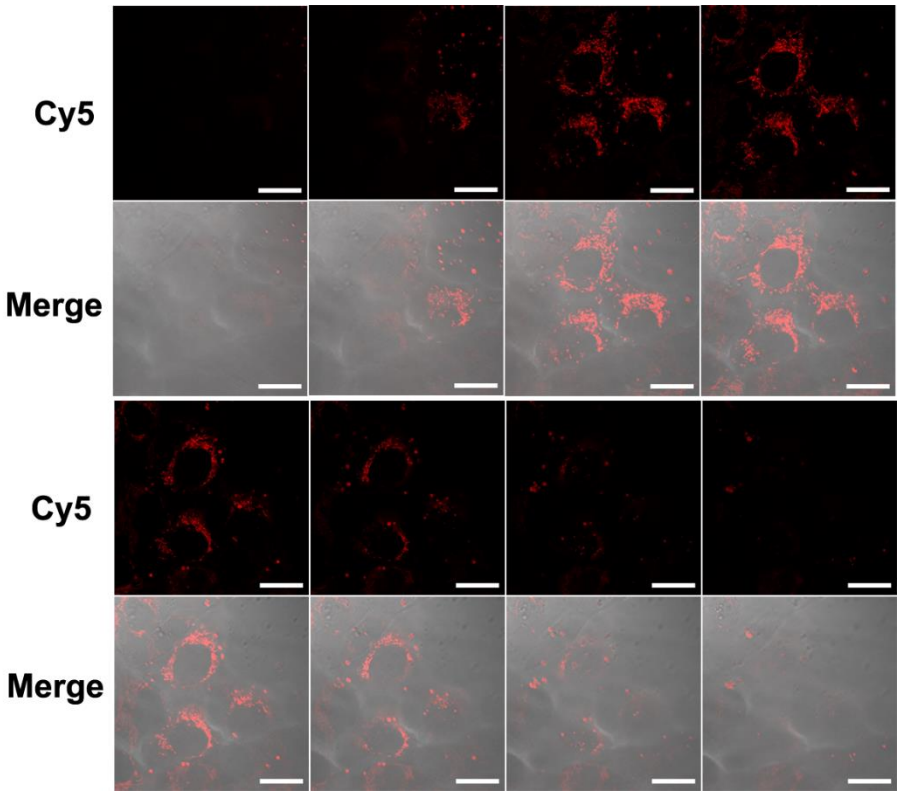


**Figure S8**. Z-stack images of HeLa cells with NaA43 DzCHA probe. Scale bar: 20 μm.

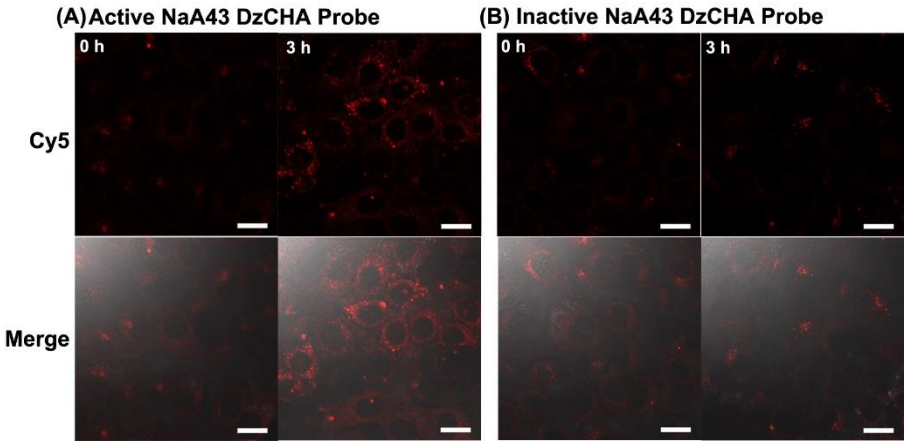


**Figure S9.** Confocal microscopy images of cells transfected with active caged NaA43 DzCHA probe (A) and inactive caged NaA43 DzCHA probe (B). Cells were first transfected with G8 cationic polypeptide for 4h, then irradiated with UV light at 365 nm for 30 min. The images showed the fluorescence signals of the sensor inside cells before and after 3 h incubation. Scale bar: 20 μm.

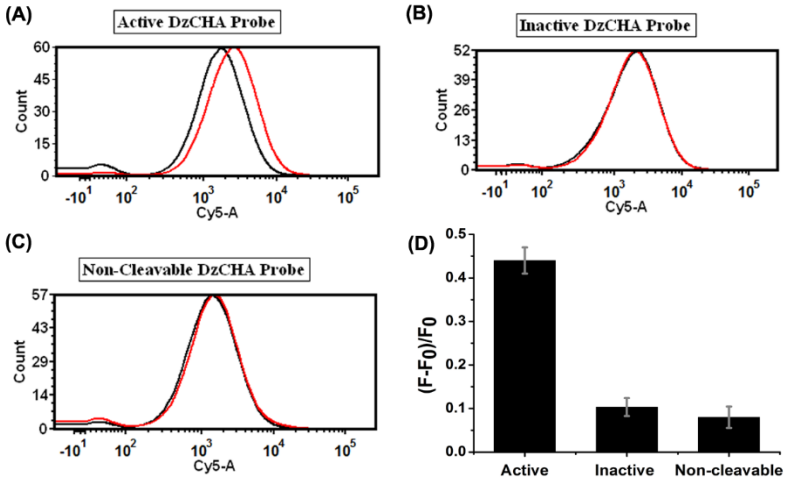


**Figure S10**. Flow cytometry assay of HeLa cells transfected with active caged DzCHA probe (A), inactive caged DzCHA probe (B) or non-cleavable DzCHA probes (C). Black lines represented samples assayed immediately after irradiation at 365 nm for 30 min and red lines represented samples incubated for further 3 h after irradiation. (D) The mean fluorescence intensity enhancement of these three kind of probes. Error bars represented three independent experiments.

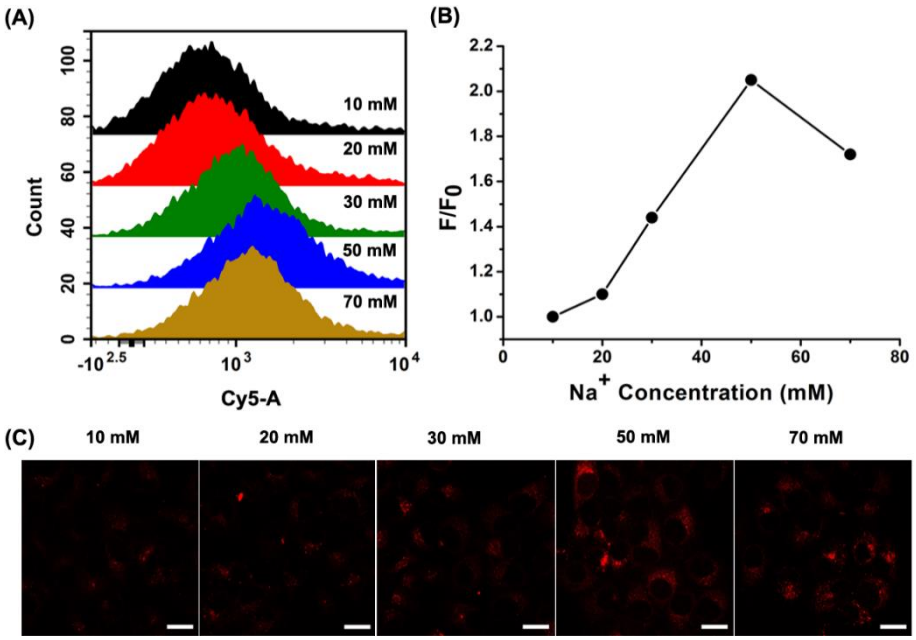


**Figure S11.** (A) Flow cytometry profiles of DzCHA probe transfected Hela cells in response to various $Na^+$ concentration. From top to bottom, the concentrations of sodium ions were 10 mM, 20 mM, 30 mM, 50 mM and 70 mM, respectively. (B) Plot of ratios for peak intensity of flow cytometry profiles (with reference to the profile for 10 mM $Na^+$) versus $Na^+$ concentrations. (c) Confocal microscopy images of DzCHA probe transfected Hela cells in response to various $Na^+$ concentration. From left to right, the concentrations of sodium ions were 10 mM, 20 mM, 30 mM, 50 mM and 70 mM, respectively. It was noteworthy that cells with influx of 70 mM $Na^+$ showed decreased fluorescence, which might be attributed to the damage of cells at high $Na^+$ concentration, concomitant with efflux of DzCHA probe.