Electronic Supplementary Information

Molecular beacon-based DNA switch for reversible pH sensing in vesicles and live cells

Nagarjun Narayanaswamy,[a] Raji R. Nair,[b] Y. V. Suseela,[a] Deepak Kumar Saini,[b] and T. Govindaraju*[a]

[a]Bioorganic Chemistry Laboratory, New Chemistry Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Jakkur P.O., Bengaluru 560064, India.

E-mail: tgraju@jncasr.ac.in

[b]Department of Molecular Reproduction, Development and Genetics, Indian Institute of Science, Bengaluru 560 012, India.

Materials and Methods

***General information*.** HPLC purified oligonucleotides (LMB, UMB and CMB), L-α-phosphatidylcholine, and phosphate buffered saline (Na-PBS) were purchased from Sigma–Aldrich. All other reagents were used as received unless otherwise mentioned. UV-vis, fluorescence and circular dichroism (CD) spectra of samples were analyzed in quartz cuvette of 10 mm path length.

Sample preparation for UV-vis, emission and CD measurements. DNA stock solutions were prepared by dissolving oligo samples in double-distilled (dd) water in the order of 10^{-4} M. Solutions of DNA duplexes were prepared in phosphate buffer solution (10 mM, pH = 7) buffer solution by mixing complementary DNA strands in equimolar concentration; this solution was then subjected to annealing by heating up to 85 °C for 15 min., subsequently cooled to room temperature for 7 h and stored in the refrigerator (4 °C) for 4 h.[1]

UV-vis absorption, emission and CD spectroscopy. The UV–vis absorption and emission spectra were recorded on Agilent Technologies Cary series UV-vis-NIR absorbance and Cary eclipse fluorescence spectrophotometers, respectively. CD measurements were carried out on Jasco J-815 spectrometer equipped with a Peltier-type temperature controller (CDF-4265/15) under a nitrogen atmosphere to avoid water condensation. Scans were performed over the range of 200-400 nm with a speed of 100 nm/min, and the spectra represent an average of three scans. A blank sample containing phosphate buffer solution (10 mM, pH = 7) was treated in the same manner and subtracted from the collected data.

HeLa cells maintenance. Human cervical carcinoma cell line (HeLa) was cultured in DMEM (Dulbecco′s Modified Eagel′s Medium) with 10% FBS (Fetal Bovine Serum). The antibiotics pencilin and streptomycin (1%) was mixed with 10% FBS medium. The cells were incubated at

37 °C temperature and 5% CO_2 humidified chamber.

Live cell imaging of HeLa cells. HeLa cells were seeded overnight and treated with 100 nM LMB for 8 hours prior to live cell imaging. Images were acquired using Olympus IX 83 inverted epifluorescence microscope using a 20X objective in Cy3 and Cy5 imaging channels. Just before imaging pH of the solution was adjusted to 3 using aq. HCl in 1X PBS.

Detection of LMB using fluorescence plate reader. The cells were incubated with LMB for 8 h inside incubator, washed with PBS and analyzed to detect LMB dye fluorescence using Infinite M1000 Pro, Tecan, Austria. Wavelengths used for excitation and emission for Cy3 was 512 nm-550 nm, Cy5 channel 625 nm-670 nm and FRET channel 512 nm-670 nm excitation-emission wavelengths.

Transfection of cells using LMB. The cells were transfected with LMB sensor using Turbofect transfection reagent (ThermoFisher Scientific Inc., USA) as per the manufacture's protocol.

Trypan Blue exclusion assay for cell viability. The cells were loaded with 1× Trypan blue solution and imaged using 10X objective with an inverted IX81 microscope, equipped with DP72 colour CCD camera (Olympus, Japan). The images were acquired to discriminated between live and dead cells.

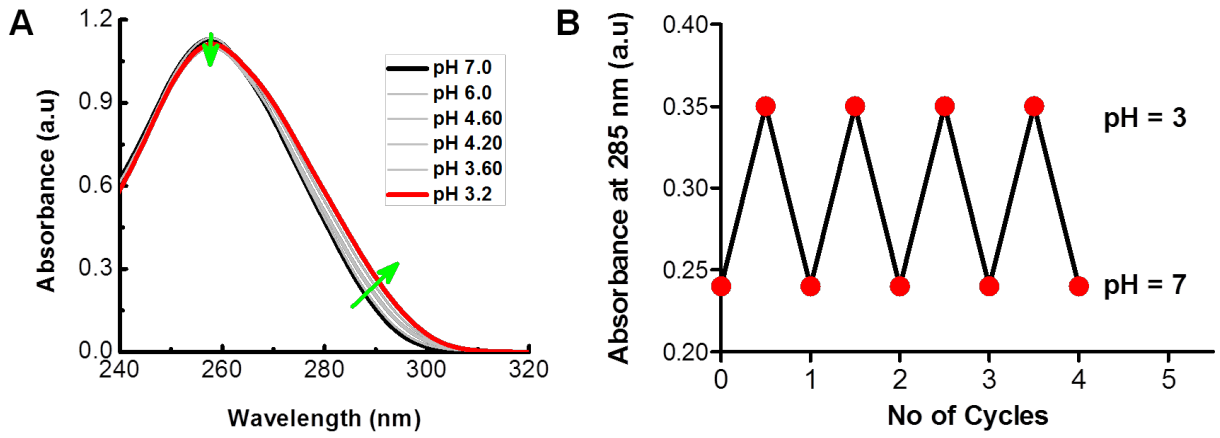

Fig. S1 (A) Absorption spectra of UMB (4 μM) with decreasing pH from 7 to 3. (B) Graph represents the structural transformation of UMB (4 μM) from the closed state to open state by alternative addition of acid and base for several cycles in phosphate buffer solution (Na-PBS, 10 mM).

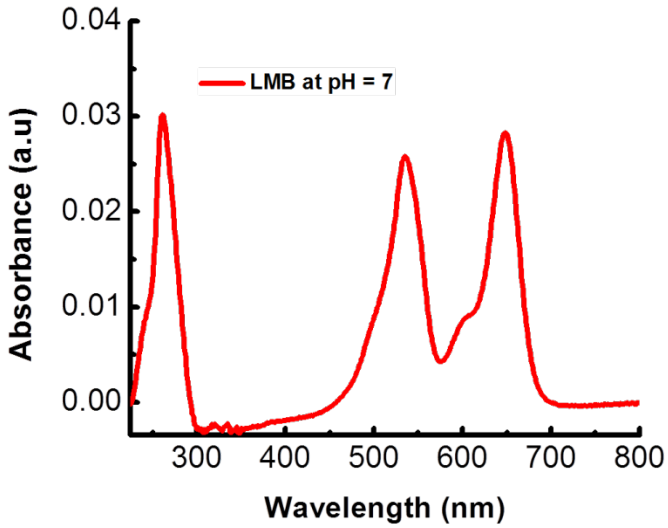

Fig. S2 Absorption spectra of Cy3 and Cy5 labeled DNA-switch (LMB) (160 nM) in phosphate buffer solution (Na-PBS, 10 mM, pH = 7).

FRET efficiency between Cy3 and Cy5 dyes. Stock solutions of Cy3 and Cy5 labeled DNA-switch (LMB) of 30 μM and unlabeled complementary DNA-switch (CMB) of 300 μM were prepared by dissolving in MQ-water solution. Stock solution of DNA-switch (LMB) was diluted to 160 nM with buffer solution in all the fluorescence measurements. Fluorescence spectra of DNA-switch (LMB) were measured upon excitation at 530 nm and emission was collected from 545-800 nm in absence and presence of complementary DNA-switch (CMB) (10 eq.). FRET efficiencies were calculated from the following formula,

$$E = 1\text{-}I_{DA}/I_D = 1/[1+(R/R_0)^6]$$

Where, I_D is intensity of Cy3 in presence of 10 eq. of complementary DNA-switch (CMB) to make sure that DNA-switch exists in open state, I_{DA} is intensity of Cy3 in presence of Cy5, R_0 is förster's distance and R is the intrafluorophore distance.

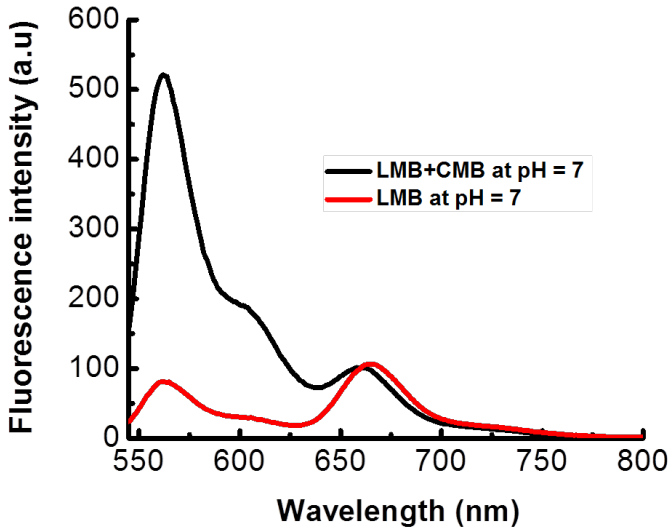

Fig. S3 Emission spectra of Cy3 and Cy5 labeled DNA-switch (LMB) (160 nM) in absence and presence of 10 eq. of unlabeled complementary DNA-switch (CMB) in phosphate buffer solution.

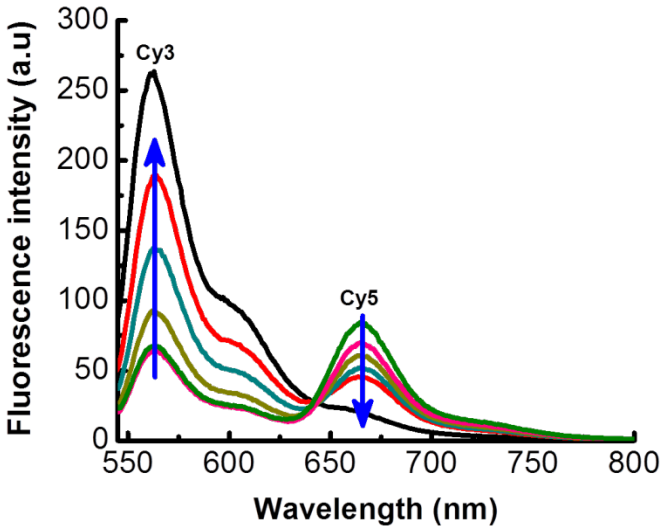

Fig. S4 Emission spectra of Cy3 and Cy5 labeled DNA-switch (LMB) (160 nM) by varying pH from 7 to 3 in phosphate buffer solution.

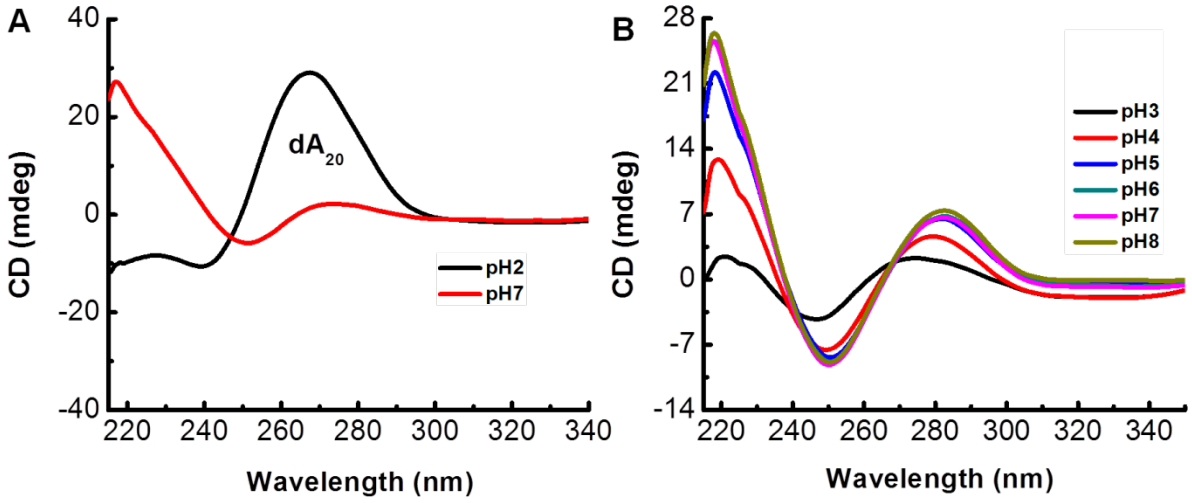

Fig. S5 (A) CD-spectra of dA_{20} (4 μM) at neutral pH = 7 and acidic pH = 2. (B) CD-spectra of unlabeled DNA-switch (UMB) (4 μM) by varying pH from 7 to 3 in phosphate buffer solution.

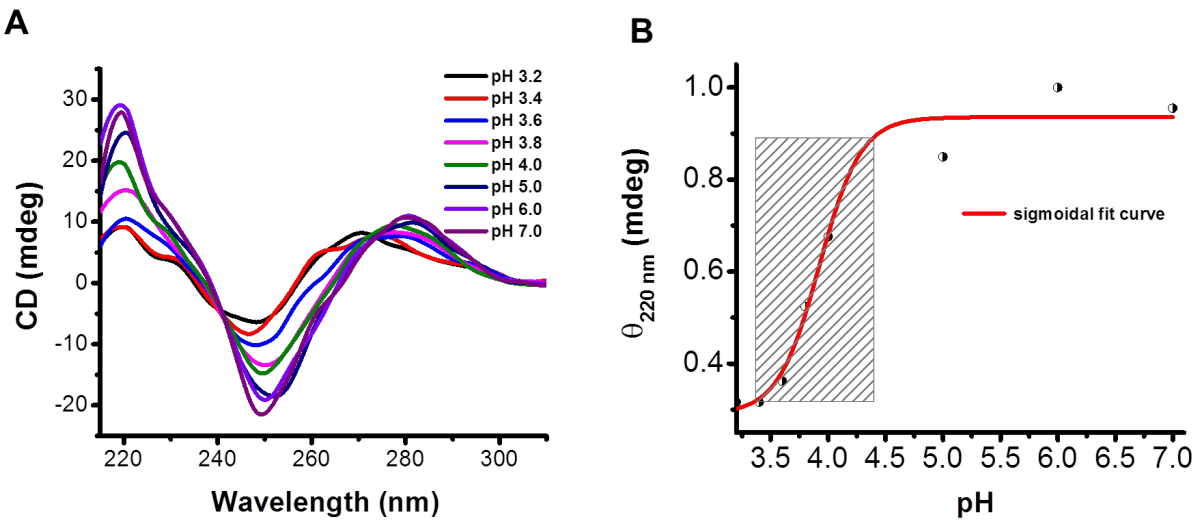

Fig. S6 (A) CD-spectra of unlabeled DNA-switch (UMB) (4 μM) by varying pH from 7 to 3 with 0.2 pH units difference from 3 to 4 in PBS. (B) Normalized sigmoidal curve showing pH dependence of CD signal of UMB at 220 nm and exponential increase in the pH region of 3.4 to 4.5.

Preparation of vesicles. Vesicles were prepared following inverted emulsion method reported in literature.[2] In brief we have described below.

Lipid-dodecane mixture: L-α-phosphatidylcholine (PC) (2 mg) was taken in a sample vial (8 mL) and dichloromethane (1 mL) was added. A thin film of lipid was made by flushing with nitrogen and kept in high vacuum for 40 min and vacuum was released under nitrogen atmosphere. Then, 5 mL of dodecane was added to thin film and sonicated for 30 min. After sonication, lipid-dodecane mixture was heated up to 50 °C for 3h and cooled to room temperature, stored at 4 °C.

Mixture A: A 100 μL of lipid-dodecane mixture was slowly placed on the top of external buffer (200 μL) for the formation of lipid mono layer under ice cooled temperature for 2h.

Mixture B: 5 μL of LMB containing buffer solution [Internal buffer (**IB**)] was added to lipid-

dodecane solution (600 μL) and sonicated for 30 min to form the emulsion. The obtained emulsion was stored at 4 °C for 15 min.

Next, DNA-switch (LMB) containing mixture B (200 μL) was added slowly on the top of mixture A and incubated at 4 °C for 15 min. Then, above mixture was centrifugated at 4 °C for 12 min with 2000 rpm, followed by 5 min with 4000 rpm and 4 min with 6000 rpm. After centrifugation, upper dodecane layer was removed carefully with pipette. The remaining solution contains the vesicles in external buffer solution.

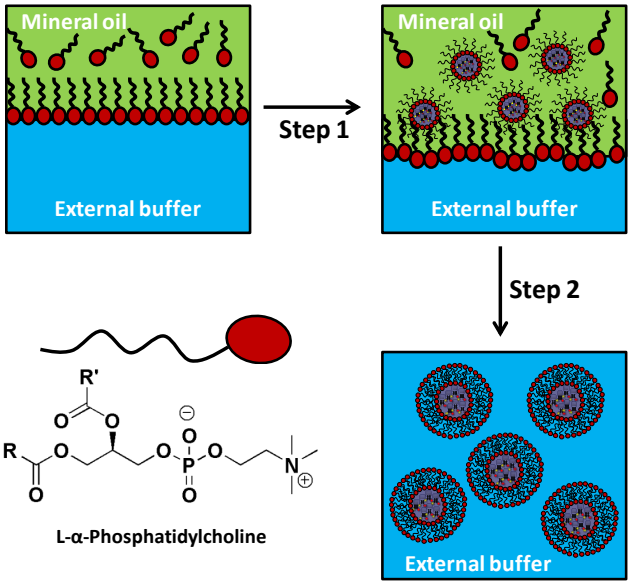

Fig. S7 Schematic view of preparation of vesicles from an inverted emulsion method.

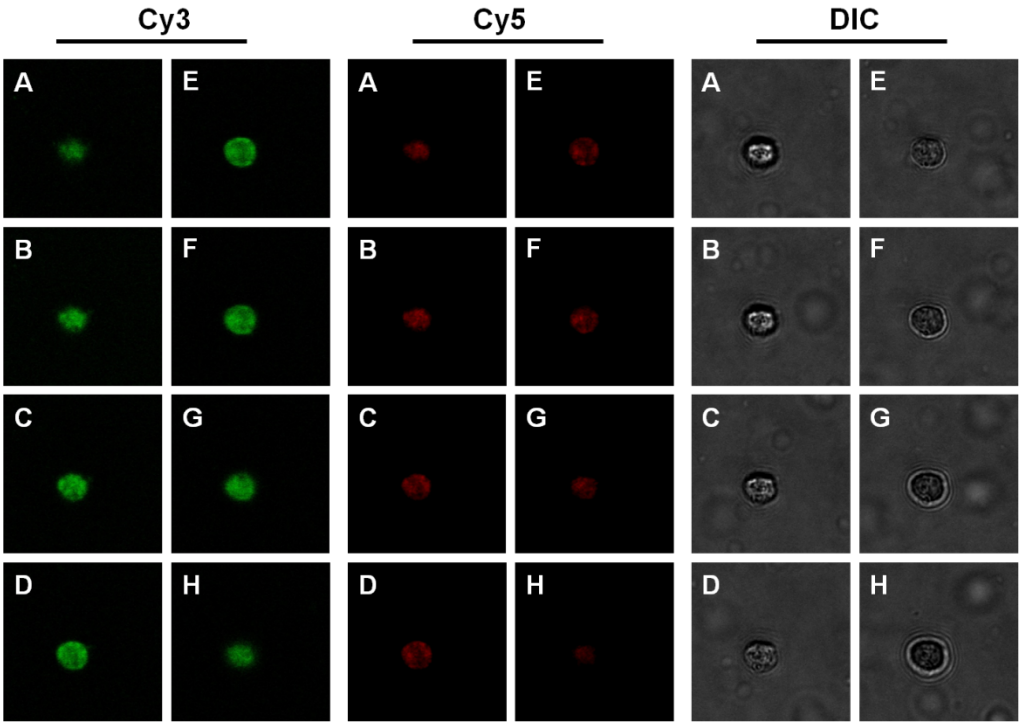

Fig. S8 Fluorescent confocal micrographs with a cross sectional analysis showing the encapsulation of labeled DNA-switch (LMB) inside the vesicles not on the walls.

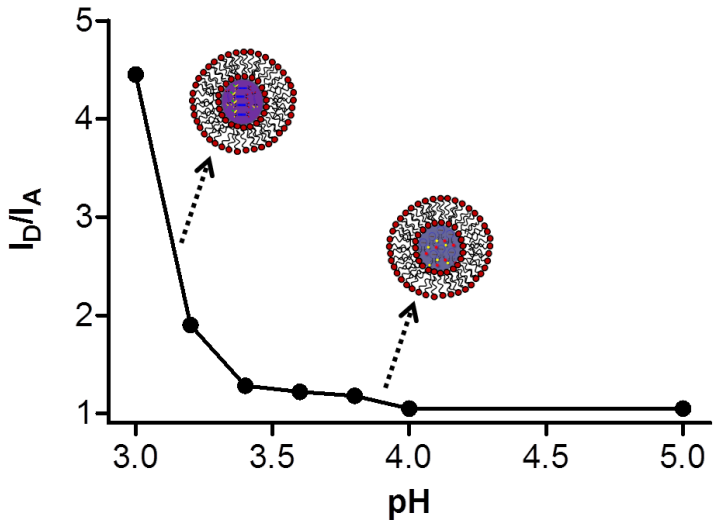

Fig. S9 Fluorescence intensity ratio's of donor vs. acceptors (I_D/I_A) of LMB encapsulated in vesicles by varying the solution pH from 5 to 3.

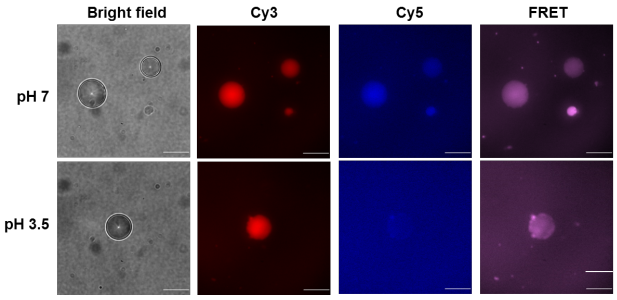

Fig. S10 Fluorescence microscope images of vesicles at solution pH 7 and 3.5.

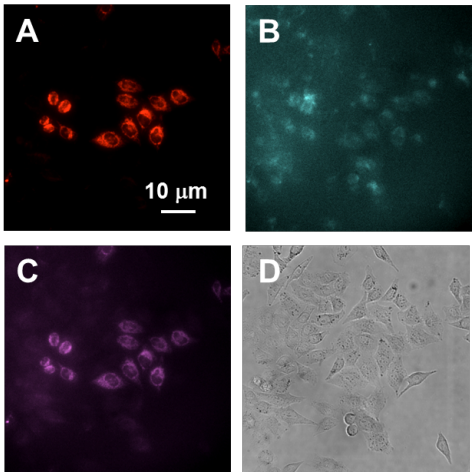

Fig. S11 (A-D) Epifluorescence microscopy images of HeLa cells upon incubation with LMB, (A) Cy3; (B) Cy5; (C) FRET; (D) bright field image (DIC).

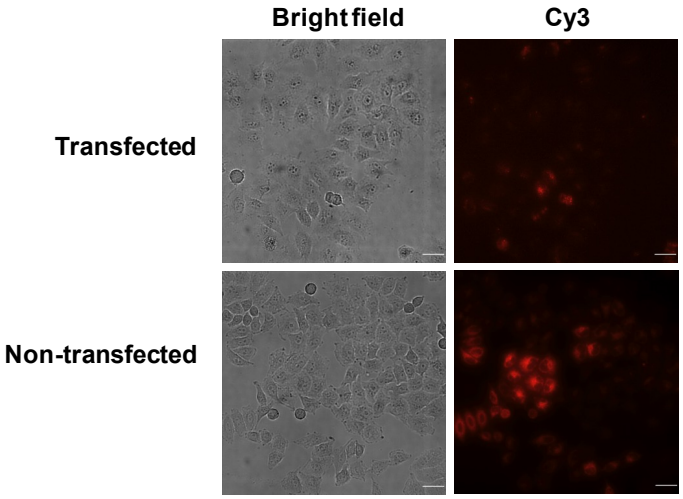

Fig. S12 LMB sensor is spontaneously taken up by HeLa cells. Epifluorescene imaging of HeLa cells transfected with LMB sensor (upper panel) and upon incubation with LMB for 8 h (lower panel), Scale = 40 μm.

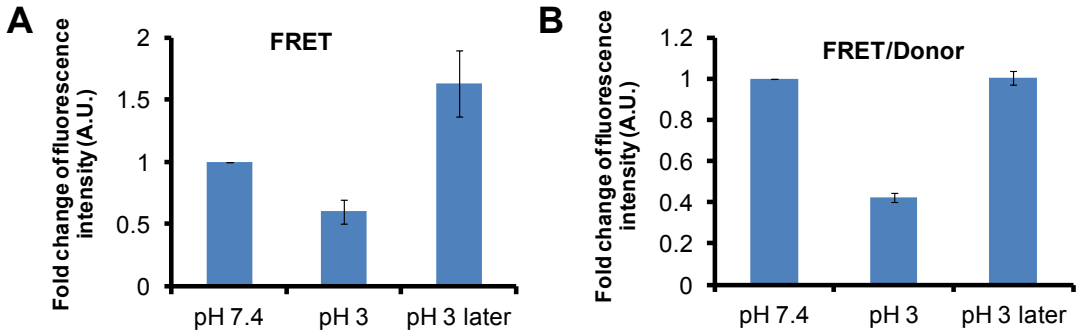

Fig. S13 LMB sensor reports dynamic buffering capacity of living cells. Change in fluorescence intensity of LMB in HeLa cells at pH=7.4 and 3, cells exposed to pH 3 for prolonged time (more than 30 min for intracellular buffering to act). (A) The left panel reports only the FRET recording (excitation 512 nm and emission 670 nm). (B) Right panel shows the FRET (donor excitation acceptor emission)/ DD ratio (donor excitation donor emission).

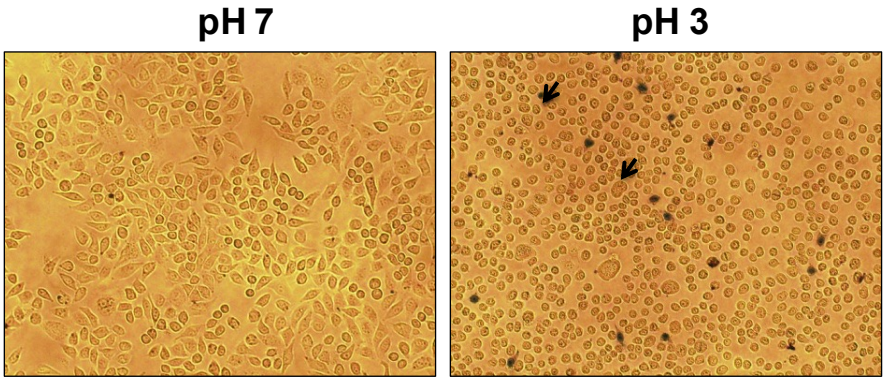

Fig. S14 Sensitivity of cells to various pH. Bright field images for Trypan Blue exclusion assay performed on HeLa cells exposed to pH 7.4 or 3. Dead cells show the uptake of dye visualized as blue colored cells (arrows).

References

1. N. Narayanaswamy, M. Kumar, S. Das, R. Sharma, P. K. Samanta, S. K. Pati, S. K. Dhar, T. K. Kundu and T. Govindaraju. *Sci. Rep*., 2014, **4**, 6476.
2. (a) S. Pautot, B. J. Frisken and D. A. Weitz, *Langmuir*, 2003, **19**, 2870-2879; (b) A. Saha, G. Mondal, A. Biswas, I. Chakraborty, B. Jana and S. Ghosh, *Chem. Commun.*, 2013, **49**, 6119–6122.