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Application of fluorescence lifetime imaging of enhanced green fluorescent protein to intracellular pH measurements

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Material and methods

Cell preparation

HeLa cells were grown in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum, 1×10^5 U/L penicillin G, and 100 mg/L streptomycin sulfate at 37°C in a humidified atmosphere containing 5% CO_2 at 37°C. HeLa cells were transfected in LAB-TEK 8-well chambered coverslips (Nalge Nunc International). Cells were transfected with plasmid DNA of pEGFP-C1 (Clontech, Palo Alto, CA) and Optifect (Invitrogen) according to the manufacturer's protocol. The DNA for transfection was prepared using endotoxin free column chromatography (Qiagen, Chatsworth, CA).

FLIM measurement with different pH

EGFP-expressed HeLa cells grown on LAB-TEK chambered coverslips with eight wells were washed with Opti-MEM I reduced serum medium and then incubated with media (5 mM glucose, 125 mM KC1, 20 mM NaC1, 10 mM HEPES, 10 mM MES, 0.5 mM $CaCl_2$, 0.5 mM $MgCl_2$) containing 25μM monensin at different pH.[1,2] Monensin is a kind of Na^+/H^+ ionophore, which equilibrates protons across plasma membrane. Monensin was diluted to a final concentration of 25 μM from the 10-mM stock solution in ethanol.

Measurements of fluorescence lifetime image

Measurements of fluorescence lifetime image were carried out using a time-correlated single-photon counting method and a four-channel time-gated detection system.[3–5] A Tsunami mode-locked Ti:sapphire laser (Spectra Physics) pumped by a Millennia Xs diode laser (Spectra Physics) was used as the excitation light source. The pulse duration and the repetition rate of the laser pulse were 80 fs and 81 MHz, respectively. The second harmonic of an ultrafast

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harmonic system (Inrad) was used for excitation. The excitation beam was coupled to a single-mode optical fiber by a fiber coupler (Five Lab) and was introduced into the scanner head (Nikon) of a TE2000-E confocal microscope (Nikon). The excitation beam was focused onto the sample with a 40× or 60× oil objective, and the fluorescence from the sample was collected with the same objective and transmitted into the scanner head, followed by a filter box equipped with an interference filter (SIGMA-KOKI) to eliminate scattered excitation light. Fluorescence was detected using a pulse counting photomultiplier in a LIMO high-speed lifetime imaging module (Nikon Europe BV).

Fluorescence decay was measured for each pixel of the confocal microscope image. To minimize the amount of data generated, the lifetime imaging module captures the fluorescence decay trace into four time windows using the time-gating electronics (Fig. S1). Each reference trigger of the laser-pulse train enables four accumulation registers sequentially, and the detected fluorescence photons are counted and accumulated by one of the four accumulation registers. Each fluorescence lifetime was evaluated by analyzing the four time-window signals assuming a single exponential decay to obtain the fluorescence lifetime image. The size of the image was 256×256 pixels. The diameter of the pinhole was 100 μm. All of the time windows were set at 2.0 ns. The background was evaluated by the counts at the area where fluorescent cells were not observed. The compensation of the delay of the fluorescence photon signals was adjusted by measuring the fluorescence lifetime images of a standard slide (Molecular Probes) or dye molecules in film.

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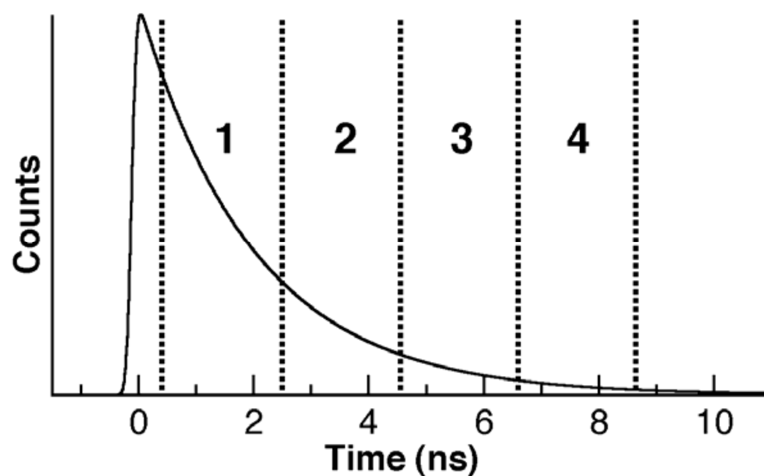

Fig. S1 Schematic illustration of the four-channel time window detection.

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Supporting references

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