

Supporting Information
for
**Ratiometric detection of pH fluctuation in mitochondria with a new
fluorescein/cyanine hybrid sensor**

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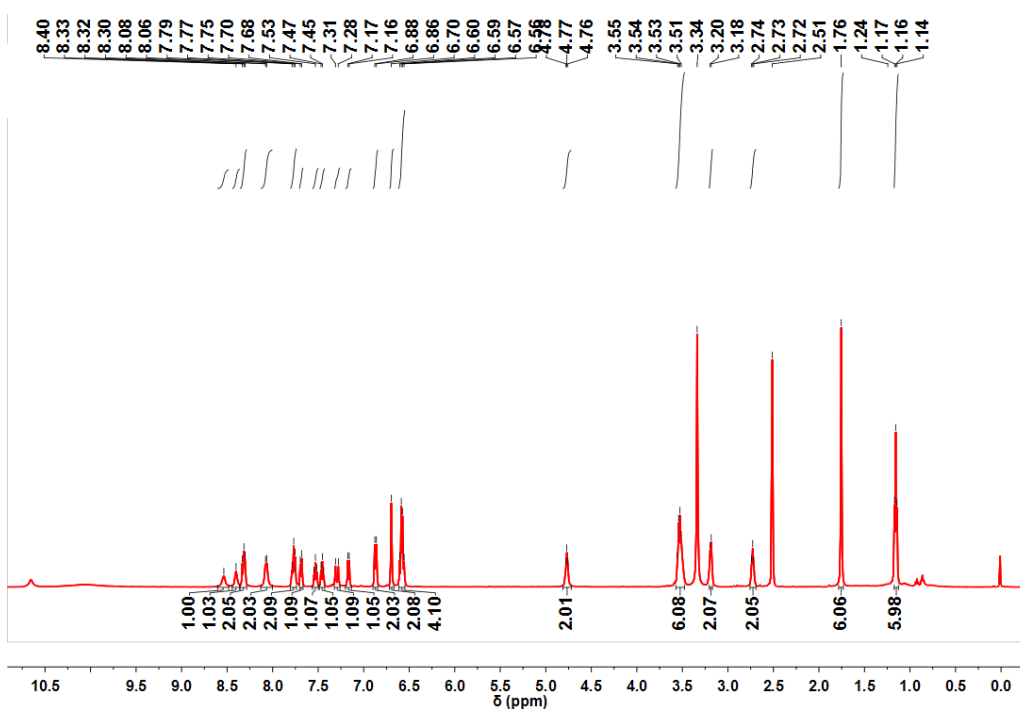


Fig. S1 ^1H NMR spectrum of Mito-pH in $\text{DMSO-}d_6$.

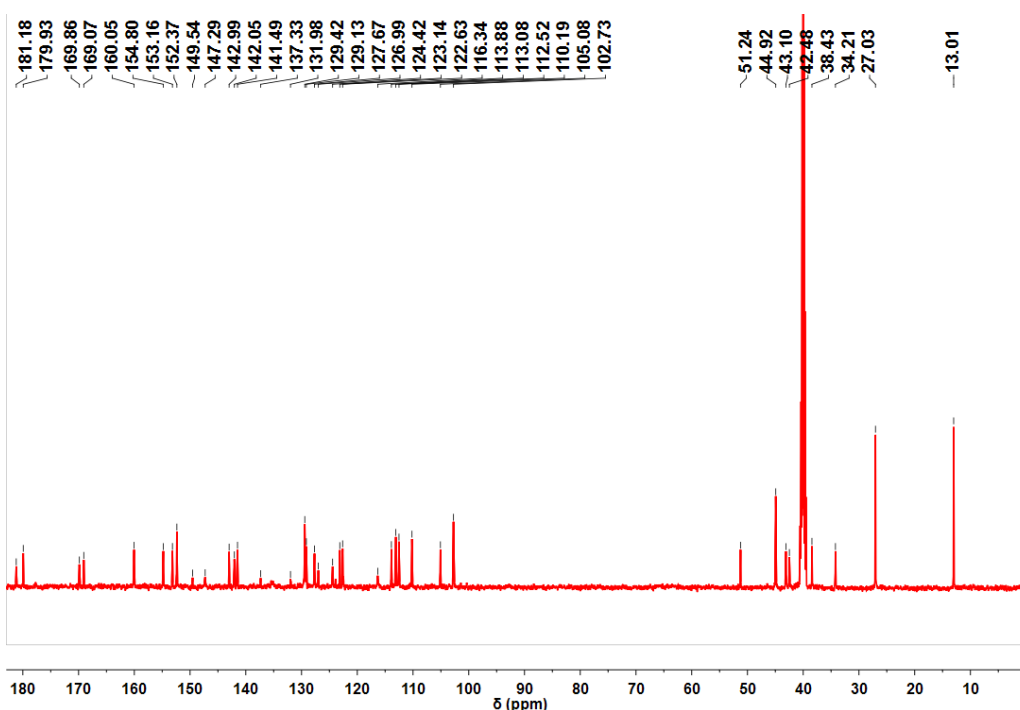


Fig. S2 ^{13}C NMR spectrum of Mito-pH in $\text{DMSO-}d_6$.

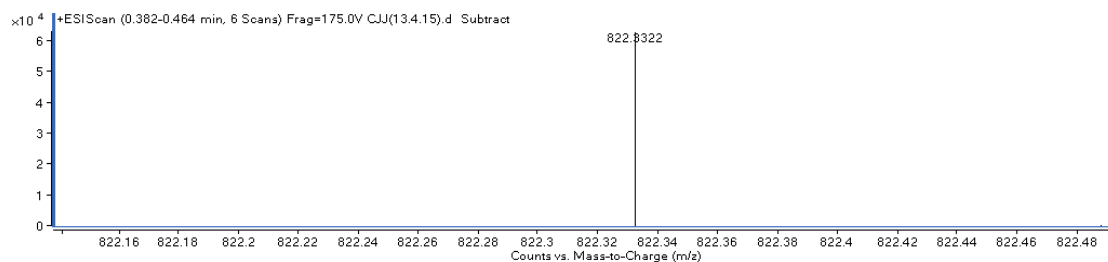


Fig. S3 ESI-HRMS of Mito-pH.

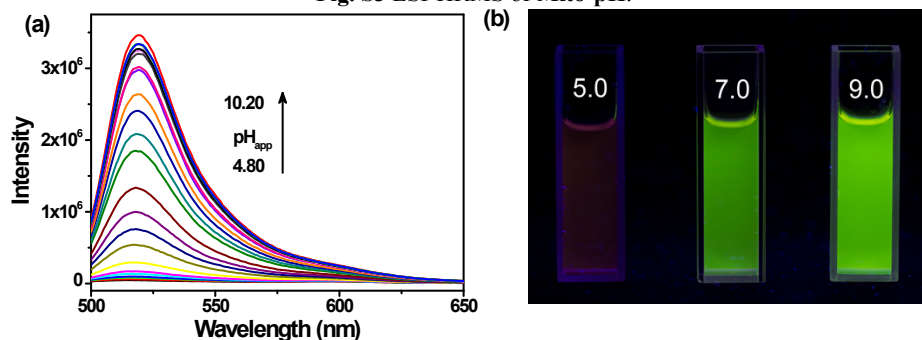


Fig. S4 (a) Emission spectra of 10 μM Mito-pH in PBS buffer of different pH values containing 10% DMSO(v/v). λ_{ex} , 490 nm. (b) Photograph of the fluorescence of Mito-pH in this media of different pH values upon irradiation with a UV-lamp of 365 nm.

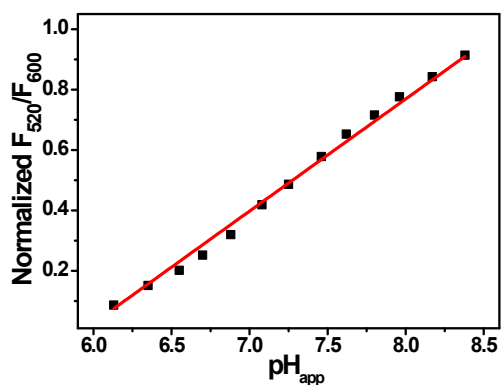


Fig. S5 Emission ratio F_{520}/F_{600} (o) of Mito-pH (10 μM) in PBS buffer of different pH_{app} values containing 10% DMSO(v/v) and its linear fitting (red line). F_{520}/F_{600} is the normalized ratio of emission at 520 nm (λ_{ex} , 490 nm) to that at 600 nm (λ_{ex} , 560 nm).

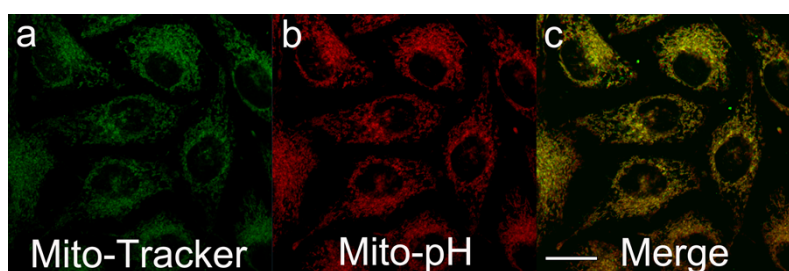


Fig. S6 Pseudo-colour confocal fluorescence images of MCF-7 cells incubated firstly with DMEM containing Mito-pH (10 μM , 60 min) and Mito-Tracker Deep Red 633 (1 μM , 30 min) at 25°C, followed by incubation with high K^+ buffers (30 mM NaCl, 120 mM KCl, 1 mM CaCl_2 , 0.5 mM MgSO_4 , 1 mM NaH_2PO_4 , 5 mM glucose, 20 mM HEPES, and 20 mM NaOAc) of pH 8.50 in the presence of 10.0 μM nigericin. (a) Fluorescence image obtained with the band path 660-750 nm upon excitation at 633 nm (Mito-Tracker channel); (b) fluorescence image obtained with the band path 560-640 nm upon excitation at 543 nm (Mito-pH channel); (c) overlay of (a) and (b). Scale bar: 20 μm .