Electronic Supplementary Information for

A Novel and Photostable pH Probe for Selectively Staining Nucleus in Living Cells

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1. Experimental section

1.1 General

All chemicals and solvents were of analytical grade and were used without further purifications. 1,2,3,3-Tetramethyl-3*H*-indolium iodide and 6-hydroxy-2-naphthaldehyde were purchased from Sigma-Aldrich company. The stock solutions of metal ions for selectivity experiments were prepared respectively by dissolving KCl, NaCl, $MgCl_2 \cdot 6H_2O$, $CaCl_2$, $Zn(NO_3)_2 \cdot 7H_2O$, $FeCl_3 \cdot 6H_2O$, $CuCl_2 \cdot 2H_2O$, $AlCl_3 \cdot 6H_2O$, $AgNO_3$, $NiCl_2 \cdot 6H_2O$, $MnCl_2 \cdot 4H_2O$, $FeCl_2 \cdot 4H_2O$, $PbCl_2$, $HgCl_2$ in doubly distilled water. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AV-400 spectrometer with tetramethylsilane (TMS) as the internal standard. The chemical shift was recorded in ppm and the following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, m = multiplet, br = broad. Mass spectra were measured on a HP-1100 LC-MS spectrometer. UV-vis spectra were recorded on Hitachi UV-3310 spectrometer. Fluorescence spectra were recorded on a Hitachi FL-4500 fluorometer. All pH measurements were performed with a pH-3c digital pH-meter (Shanghai LeiCi Device Works, Shanghai, P.R. China) with a combined glass-calomel electrode. Fluorescent images were acquired on a Nikon A1 confocal laser-scanning microscope with a 40 objective lens. The solvents used for UV-vis and fluorescence measurements are of HPLC grade.

1.2 Synthetic procedures and characterization data

1,2,3,3-tetramethyl-3*H*-indolium iodide 0.46 g (1.50 mmol) and 6-hydroxy-2-naphthaldehyde 0.29 g (1.7 mmol) were loaded in a 100 mL round bottle flask. Then, 30 ml anhydride ethanol and two drops of acetic acid was added. The reaction mixture was refluxed for 4h at nitrogen atmosphere, and then cooled down to room temperature to give large amount of crude product. The crude product was recrystallized with ethanol to give pure product as a needle red solid (0.61 g, yield: 90%). ^{1}H NMR (d^6- DMSO, 400 MHz) δ(ppm): 1.83 (s, 6H, CH_3), 4.16 (s, 3H, CH_3), 7.21-7.24 (m, 2H, Ar-H), 7.60-7.66 (m, 2H, Ar-H), 7.70 (d, *J* = 8.2, 1H), 7.86-7.93 (m, 4H, Ar-H), 8.26 (d, *J* = 4.4 Hz, 1H), 8.55 (d, *J* = 8.2 Hz,1H), 8.63 (s, 1H, Ha), 10.45

(s, 1H, OH).

^{13}C NMR (d^{6}- DMSO, 100 MHz) δ(ppm): 25.98, 52.45, 109.99, 111.91, 115.43, 120.38, 123.32, 124.99, 127.67, 129.40, 129.57, 129.77, 131.99, 135.36, 137.88, 142.36, 143.92, 154.17, 159.18, 181.99. HR-MS (ESI) calcd for $C_{23}H_{22}NO^{+}$: 328.1699, found 328.1703 for $[M]^{+}$.

1.1 equivalent of NaOH was added to HOCy in methanol, and then evaporated the solvents to give NaOCy as a purple solid.

1.3 UV-Vis and fluorescence pH titrations

50 mM PBS solutions with various pH values from 5.0 to 10.0 were prepared. Stock solution of NaOCy (2 mM) was prepared in distilled water, and then was added into the above mentioned PBS solution to prepare NaOCy solutions (20 μM) with different pH values. In the pH titrations experiments, 3 mL of NaOCy solutions (20 μM) were poured into a quartz optical cell of 1 cm optical path length each time. Excitation and emission bandwidths were both set at 5 nm, and the excitation wavelength was 448 nm. All spectroscopic experiments were carried out at room temperature.

1.4 UV-Vis and fluorescence pH titrations in serum

A series of PBS solutions with different pH values were prepared, and then were mixed with calf serum at the fraction of 50%. Finally, NaOCy was added to the solution at the concentration of 20 μM. In the pH titrations experiments, 3 mL of NaOCy solutions (20 μM) were poured into a quartz optical cell of 1 cm optical path length each time. Excitation and emission bandwidths were both set at 5 nm, and the excitation wavelength was 448 nm. All spectroscopic experiments were carried out at room temperature.

1.5 Reversibility test

100 mL NaOCy solution at the concentration of 20 μM was prepared in distilled water. The pH of NaOCy solution was carefully adjusted to 6.8 and 9.2 by 0.1 M NaOH and 0.1 M HCl. 3 mL of NaOCy solutions were poured into a quartz optical cell of 1 cm optical path length each time. Excitation and emission bandwidths were both set at 5 nm, and the excitation wavelength was 448 nm. All spectroscopic experiments were

carried out at room temperature.

1.6 Confocal fluorescence imaging

HeLa cells were cultured in RPMI 1640 medium (Invitrogen) containing 10% fetal bovine serum, penicillin (100 units/mL), streptomycin (100 mg.mL^{-1}) and 5% CO_2 at 37 ^{o}C. One day before experiment, cell suspensions were plated at a density of 1.0 x 10^4 cells/mL on 35 mm diameter round glass coverslips. Then the cells were incubated with NaOCy (10 μM) for 1h at 37 ^{o}C in 5% CO_2 -95% air and washed three times with PBS buffer (0.10 M, pH 7.40) before imaging. Fluorescent images were acquired on a Nikon A1 confocal laser-scanning microscope with a 40 objective lens.

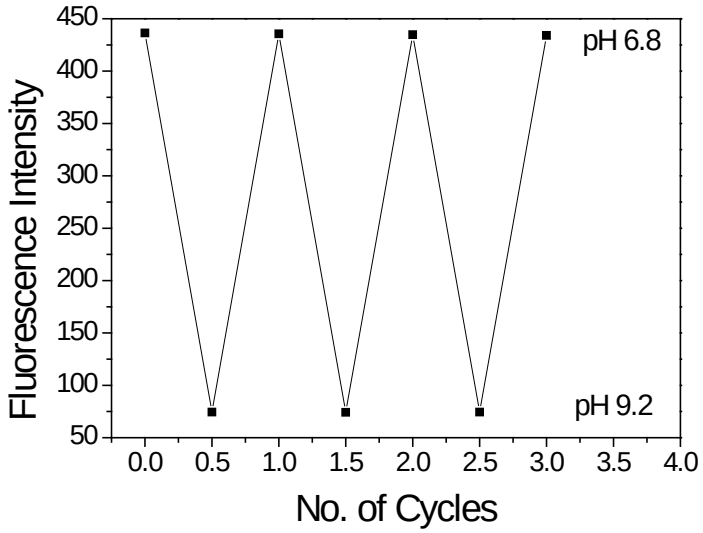

Fig. S1 The reversibility of fluorescence intensity at 565 nm for NaOCy (20 μM) between pH 9.2 and 6.8.

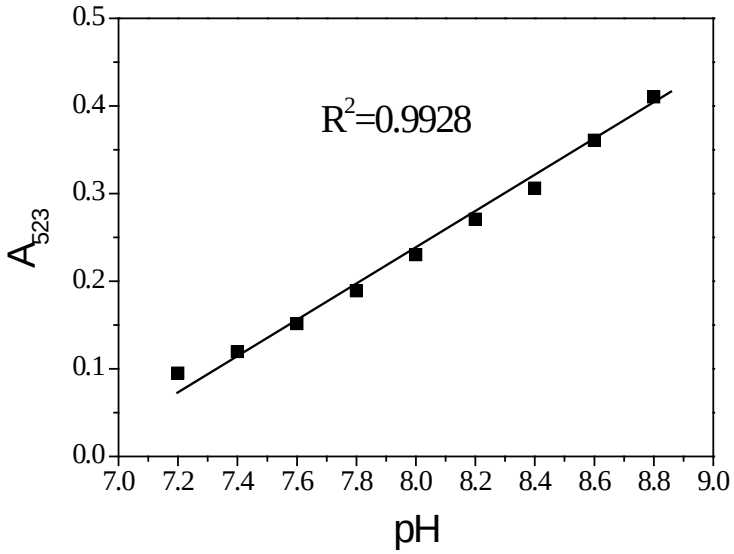

Fig. S2 A good linearity between absorbance of NaOCy (20 μM) at 523 nm and pH ranged from 7.2 to 8.8 in serum solution.

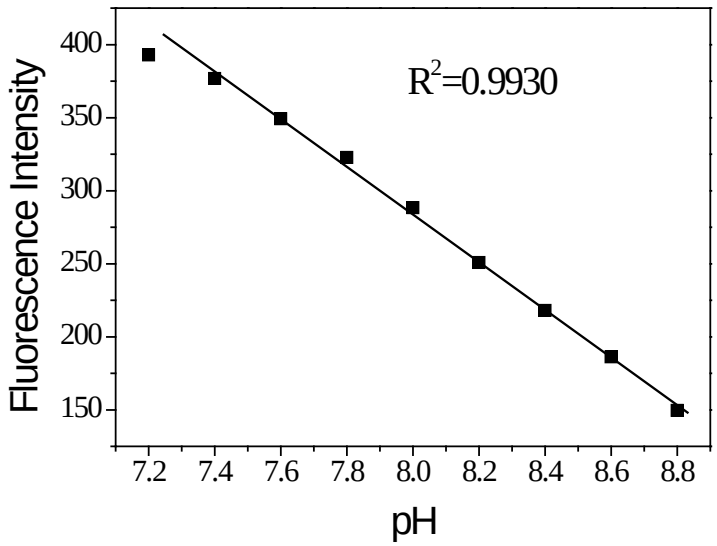

Fig. S3 A good linearity between fluorescence intensity of NaOCy (20 μM) at 565 nm and pH ranged from 7.2 to 8.8 in serum solution.

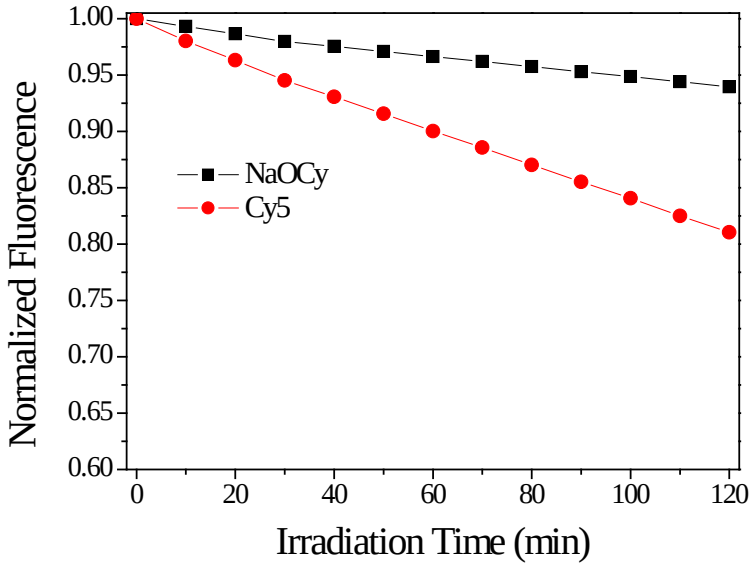

Fig. S4 Changes of fluorescence intensity (c = 20 μM in 50 mM PBS, pH =7.40) under the irradiation of 40 W Xe lamp. (a) For NaOCy, the excitation wavelength was 448 nm, and the maximum emission wavelength was 565 nm; (b) For Cy5, the excitation wavelength was 646 nm, and the maximum emission wavelength was 674 nm.

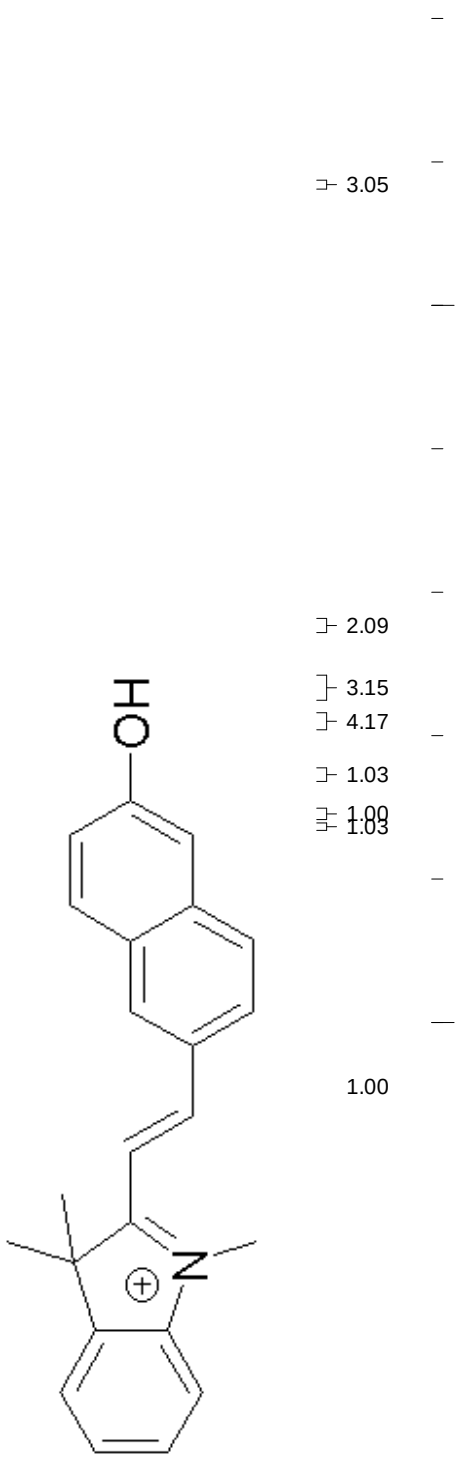

Fig. S5 ^{1}H NMR spectrum of HOCy in d^{6}-DMSO (400 M Hz)

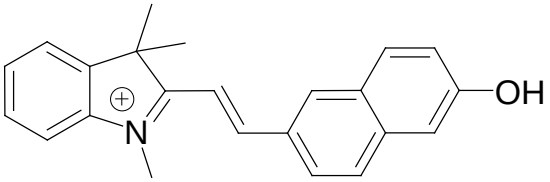

Fig. S6 ^{13}C NMR spectrum of HOCy in d^{6}-DMSO (100 M Hz)

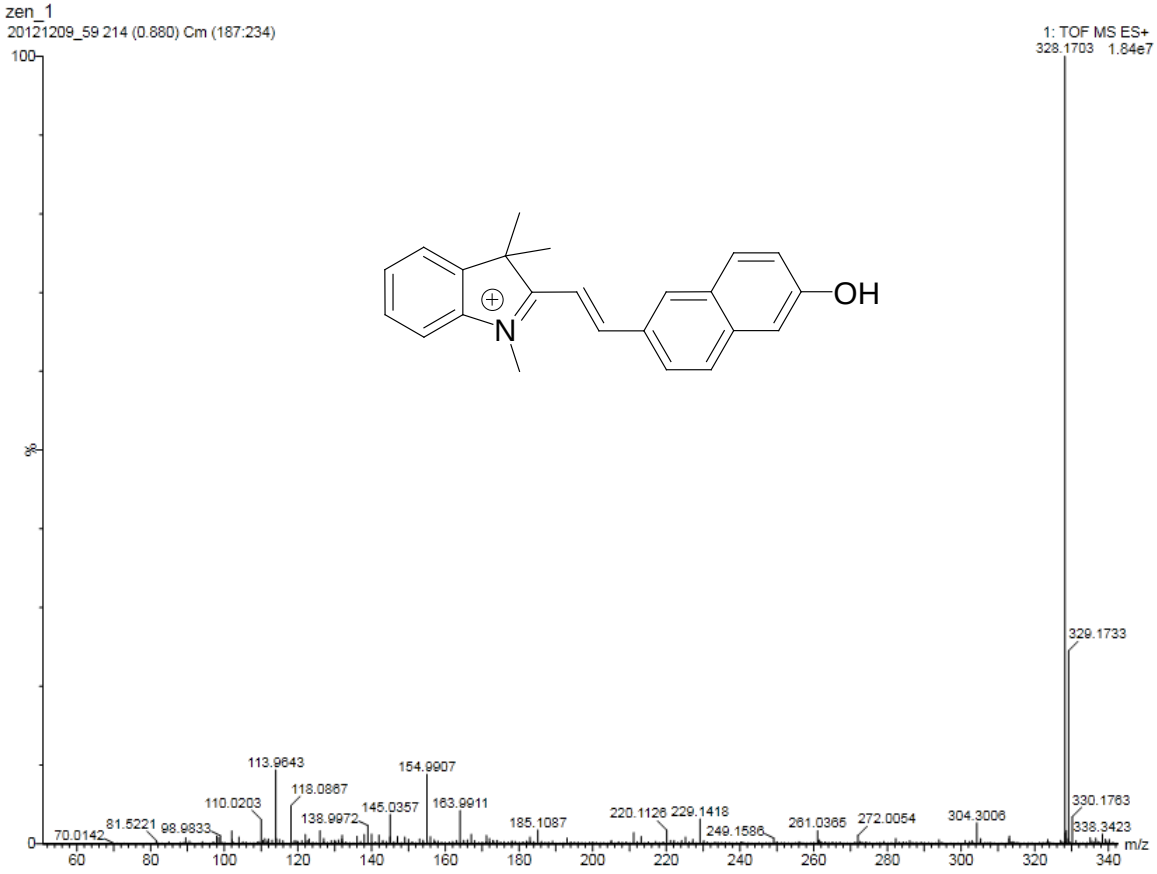

Fig. S7 HR MS (ESI) spectrum of HOCy