Electronic Supplementary Information (ESI)

Novel Nanogel-based Fluorescent Probe for Ratiometric Detection of Intracellular pH Values

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Experimental procedures and apparatus
The polyurethane hydrogel (HydroMed D4) was obtained from Cardiotech Intl. All other reagents were obtained from J&KCHEM and used without further purification. Ultrapure water (18 MΩ/cm) was used for the preparation of all aqueous solutions. Absorption spectra were recorded on Hitachi UV-3010, and the fluorescence spectra were recorded on Hitachi F-7000. Scanning electron microscopy image was obtained with an electron microscope (Hitachi S-4800). Dynamic light scattering performed on Zetasizer Nano (ZS90, Malvern). Cells were analyzed using a Confocal microscope (FV1000-IX81). NIH/3T3 Fibroblasts cells were grown at 37°C under 5% CO₂ in Dulbecco’s Modified Eagle Medium (DMEM, Gibco; Invitrogen) supplemented with 10% fetal bovine serum.

Synthesis and characterization of the pH probe
The synthesis of 8-methoxypyrene-1-carbaldehyde was completed according to the literature.1

Under argon, to a 50 mL Schlenk flask were added 8-methoxypyrene-1-carbaldehyde (100 mg, 0.4 mmol) and dry methylene chloride (10 mL). And then BBr₃ (1.2 mmol) was added slowly to the solution at 0°C, and the stirring was continued for the next 12 hours at room temperature. Finally the reaction solution
was poured into ice water, the crude extracted with dichloromethane, and the separated organic phase was dried over MgSO$_4$. The solvent was evaporated and the residue was purified by silica gel column chromatography using dichloromethane: petroleum ether=1:1 as eluent to obtain yellow powder. Yield: 80mg (90%) $^1$H NMR (400 MHz, DMSO) $\delta$ = 11.12 (s, 1H), 10.72 (s, 1H), 9.31 (d, $J$ = 9.5 Hz, 1H), 8.62 (d, $J$ = 9.5 Hz, 1H), 8.52 – 8.43 (m, 1H), 8.34 – 8.21 (m, 3H), 8.00 (d, $J$ = 8.8 Hz, 1H), 7.68 (d, $J$ = 8.3 Hz, 1H). $^{13}$C NMR (101 MHz, DMSO) $\delta$ = 193.8, 154.5, 136.4, 131.9, 131.7, 131.3, 129.4, 126.3, 125.4, 125.3, 124.7, 124.1, 124.0, 123.8, 121.2, 118.2, 114.3. HRMS (ESI) m/z [M–H]$^+$ calc. 245.05971 found: 245.06026, elemental analysis (%) calcd: C 82.91 H 4.09 ; found: C 82.71, H 4.06.

**Preparation of the composite nanogel:**

The HPC were dissolved in10 g of a 200 ppm solution of the polyurethane hydrogel (PU) in an ethanol/water (9:1, v/v) mixture. The resultant ratio of PU/HPC is 199:1 (w/w). The mixtures were stirred for 2 h, and then dialyzed against distilled water for 24 h, with an interval of 2–3 h to exchange the water. Finally, the aqueous dispersion of nanogel was filtered through a 0.22 µm filter to remove large aggregates. The resultant suspension was used in further experiments.

**Scanning electron microscopy**

![Scanning electron microscopy image of the NGIs in the lyophilized dried state.](image)

**Hydrodynamic size of the nanogel suspensions**

![Size distribution of NGIs aqueous suspension(measured via dynamic light](image)
scattering at 37 °C).

**Fig. S2** CIE 1931 (x,y) chromaticity diagram of the solution with different pH values derived from fluorescence spectra (black point); pH value from left to right: 4.0, 5.0, 6.0, 7.0, 7.2, 7.4, 7.6, 7.8, 8.0, 9.0, 10.0.

**General procedure for UV/Vis and fluorescence spectroscopy**
A series of standard pH buffers were prepared by mixing 20mM Na₂HPO₄ and 20 mM NaH₂PO₄. All pH values were measured by METTLER-TOLEDO FE20 pH-meter. The ratio signal was calculated from the fluorescence intensities at 510 nm and 610nm with λ<sub>ex</sub> = 455 nm.

**Fluorescence selectivity of NGIs**
The solution of NGIs was prepared in triple-distilled water. The biologically relevant analytes, including Na⁺(10mM); K⁺(10mM); Ca²⁺(10mM); Zn²⁺(1mM); Mg²⁺(1mM); Cu²⁺(1mM); Fe²⁺ (1mM); Fe³⁺ (1mM); Co²⁺(1mM); SO₄²⁻(10mM); NO₃⁻(10mM); Cl⁻(10mM); Gly(0.1mM); HSA(0.1mM); H₂O₂(0.1mM) were prepared and the pH value was fixed at 7.4.

**Fluorescence reversibility of NGIs**
The pH of NGIs solution (1µM) between pH 4 and pH 10 was adjusted back and forth by 2 M HCl or NaOH, and then measured by pH-meter. The fluorescence spectra were recorded with λ<sub>ex</sub> = 455 nm. All data were obtained at 37°C.

**Fig. S3** Reversible fluorescence changes of NGIs between pH 4 and pH 10.
Cell culture and fluorescence imaging

NIH/3T3 Fibroblasts cells were cultured in Dulbecco's Modified Eagle’s Medium (DMEM) supplemented with 10% FBS. The cells were plated on glass bottomed dishes and maintained in an atmosphere of 5% CO₂ at 37 °C before imaging. When labeling, the growth medium was removed and replaced with DMEM without FBS, the cells were treated with composite nanogels (1 μM of HPC) at 37 °C under 5% CO₂ for 30 min. The treated cells were washed four times with phosphate buffered saline and then cell images were obtained using a confocal microscope FV-1000-IX81 and were analyzed with FV10-ASW software.

Intracellular pH calibration

The NGIs-loaded cells were incubated at 37 °C for 15 min in high K⁺ buffer (30 mM NaCl, 120 mM KCl, 1 mM CaCl₂, 0.5 mM MgSO₄, 1 mM NaH₂PO₄, 5 mM glucose, 20 mM HEPES) with various pH values in the presence of 10 μM nigericin.

Viability assay

The cytotoxicity of NGIs was evaluated by the standard MTT assay. Briefly, NIH/3T3 Fibroblasts cells were seeded in 96-well U-bottom plates at a density of 7000 cells/well and incubated with NGIs at varied concentrations (0-10μM) at 37°C for 24 h. Then 10μL of the MTT solution (5 mg/mL in DMEM) was added to each well and followed by incubation at 37°C for another 4 h. The supernatant was removed, and 150 μL of DMSO was added to each well to dissolve the formed formazan. After shaking the plates for 15 min, absorbance values of the wells were read with a microplate reader at 490 nm. The cell viability rate (VR) was calculated according to the equation: VR = A/A₀ × 100%, where A is the absorbance of the experimental group (i.e., the cells were treated by NGIs) and A₀ is the absorbance of the control group (i.e., the intact cells).

![Fig. S4](image_url)

**Fig. S4** Effects of NGIs at varied concentrations on the viability of NIH/3T3 Fibroblasts cells.
Fig. S5 Confocal microscopy images of NGIs (1.0 μM) in NIH/3T3 Fibroblasts cells. (a) emission from the green channel; (b) emission from the red channel; (c) Bright field image of NIH/3T3 cells incubated with NGIs (d) Overlay of the Bright field, green and red channels; (All data were obtained at pH=7.4, T=37°C).

Fig. S6 In-situ fluorescence spectra of NGIs in NIH/3T3 Fibroblasts cells.

Quantitative absorption spectrum

We got the final concentration through compared absorption spectra of the nanogel of aqueous solution containing HPC and ethanol containing HPC.
References: