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Electronic Supporting Information

An indolium-based near-infrared fluorescent probe for non-invasive real-time monitoring gastric pH *in vitro* and *in vivo*

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CONTENT

1. Structural characterizations of probe Hcy-pH

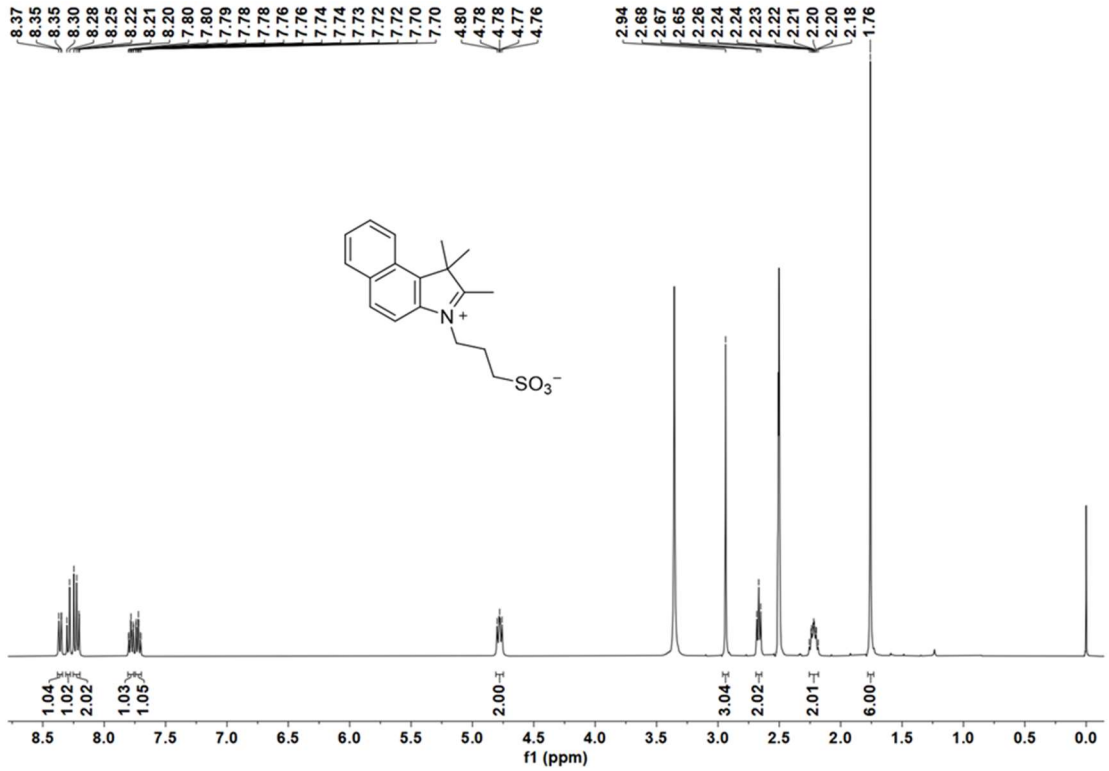

Fig. S1 ^{1}H NMR spectrum of intermediate **1** (400 MHz, DMSO-d_6).

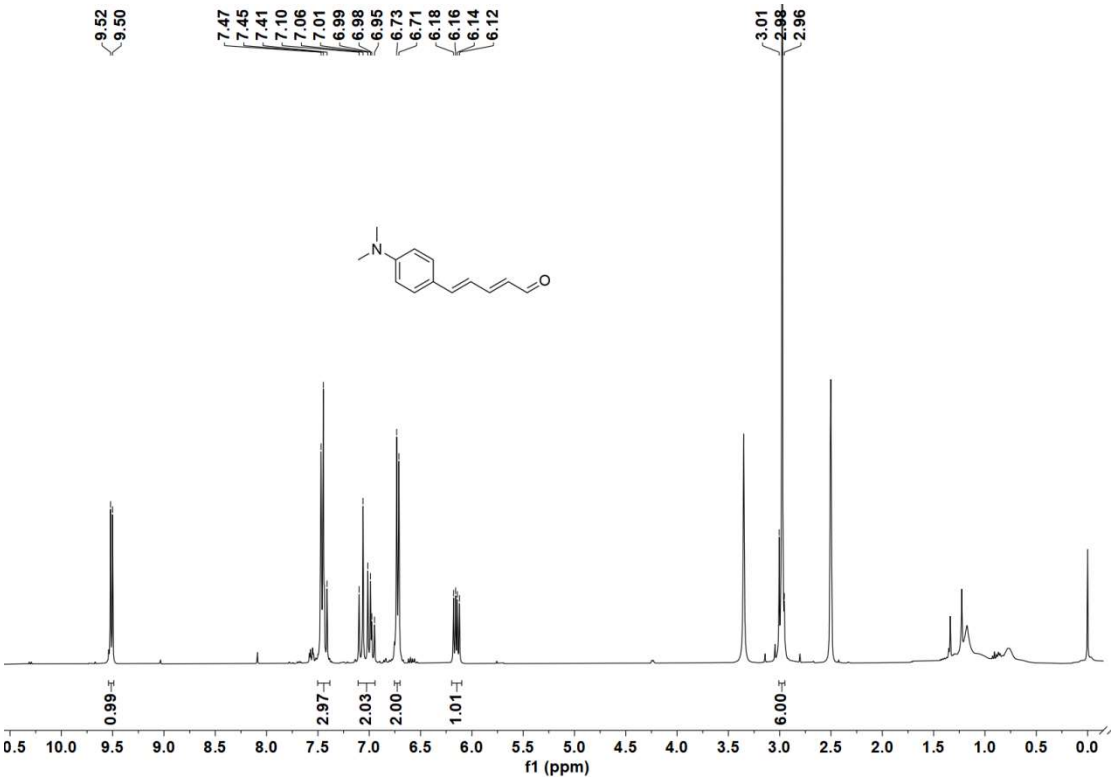

Fig. S2 ^{1}H NMR spectrum of intermediate **2** (400 MHz, DMSO-d_6).

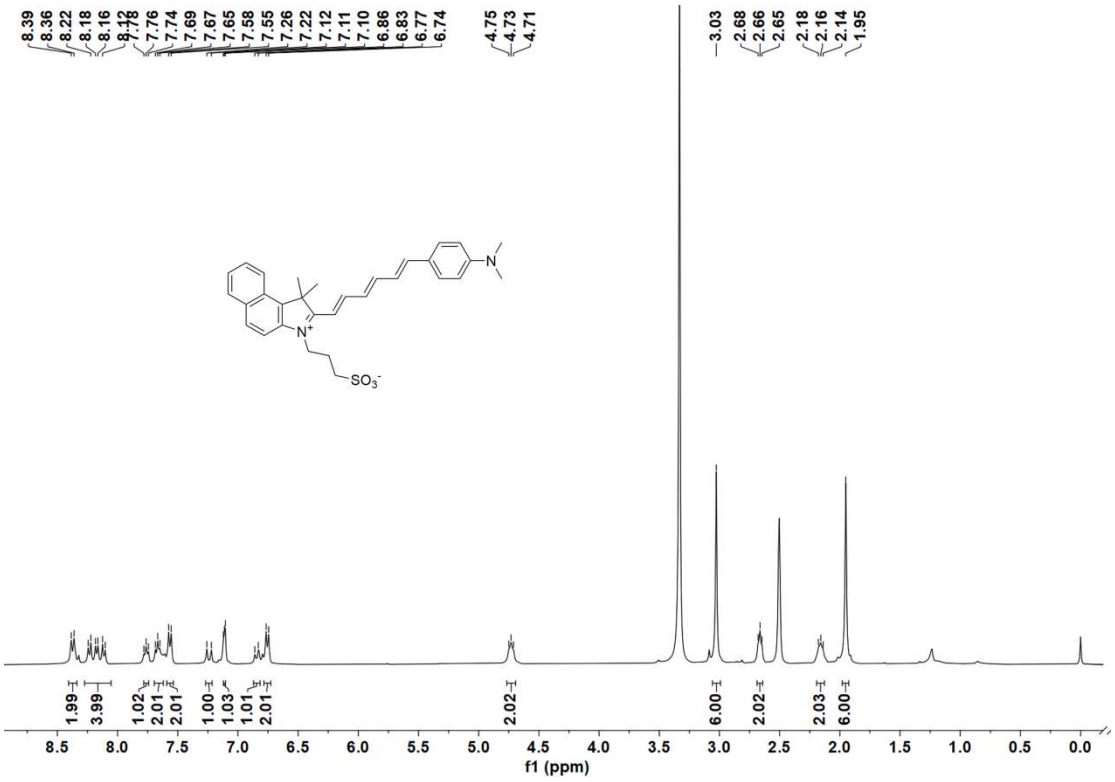

Fig. S3 ^{1}H NMR spectrum of probe **Hcy-pH** (400 MHz, DMSO-d_6).

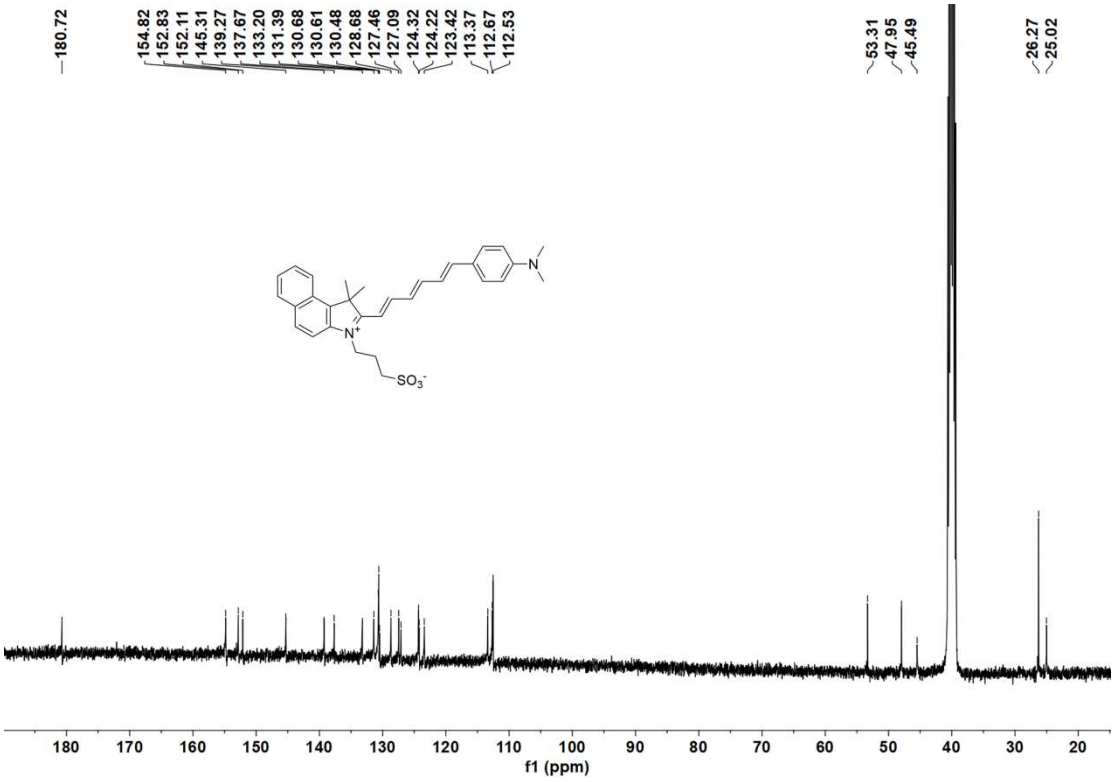

Fig. S4 ^{13}C NMR spectrum of probe **Hcy-pH** (100 MHz, DMSO-d_6).

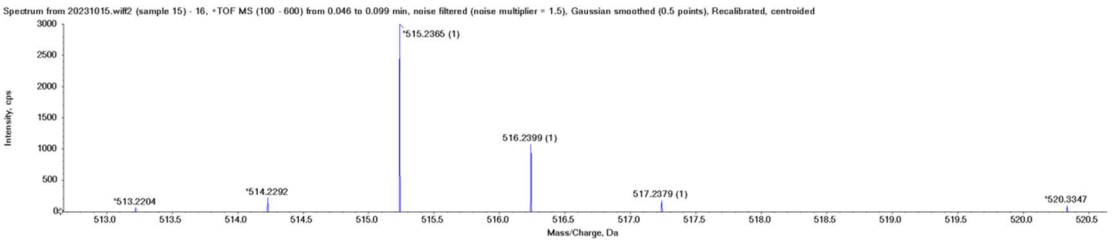

Fig. S5 HR-MS spectrum of probe **Hcy-pH**.

2. Color changes of Hcy-pH in solutions with different pH

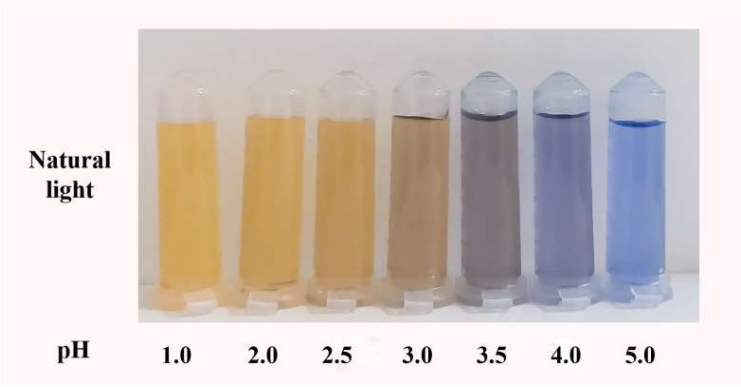

Fig. S6 The color changes of **Hcy-pH** (20 μM) in solutions with different pH values under natural light.

3. Responses of Hcy-pH to viscosity and polarity

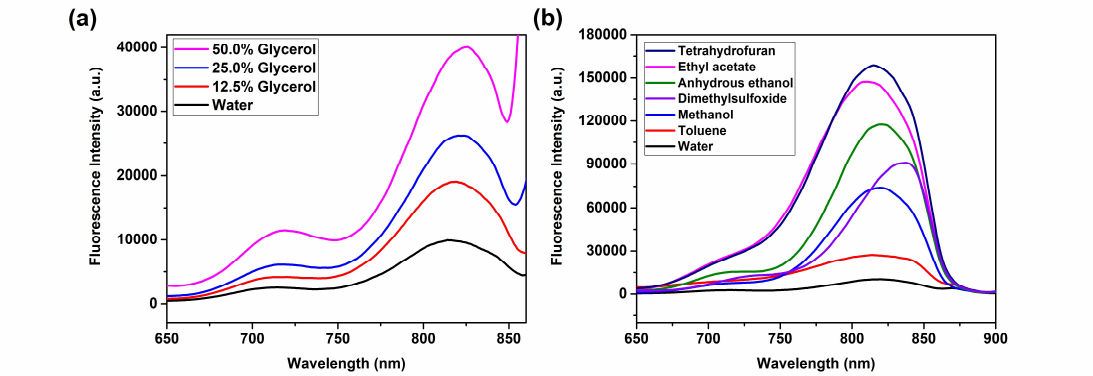

Figure S7 (a) Fluorescence emission spectra of probe **Hcy-pH** in solvents with different viscosities (pH = 7.4). (b) Fluorescence emission spectra of probe **Hcy-pH** in solvents with different polarities. $\lambda_{ex}/\lambda_{em}$ = 580/820 nm, slit width (ex/em) = 10/10 nm.

Table S1 Maximal emission wavelengths and fluorescence intensities of the probe **Hcy-pH** in different solvents.

Solvent	λ_{em} (nm)	Fluorescence intensity (a.u.)
Water	820	10376
50.0% Glycerol	820	39390.9
25.0% Glycerol	820	26626
12.5% Glycerol	820	18622
Tetrahydrofuran	810	159126.9
Ethyl acetate	810	149593.7
Anhydrous ethanol	820	119328.3
Dimethylsulfoxide	835	91183.3
Methanol	820	74587.8
Toluene	820	28256.8

4. Linear relationship between fluorescence intensity of Hcy-pH and pH

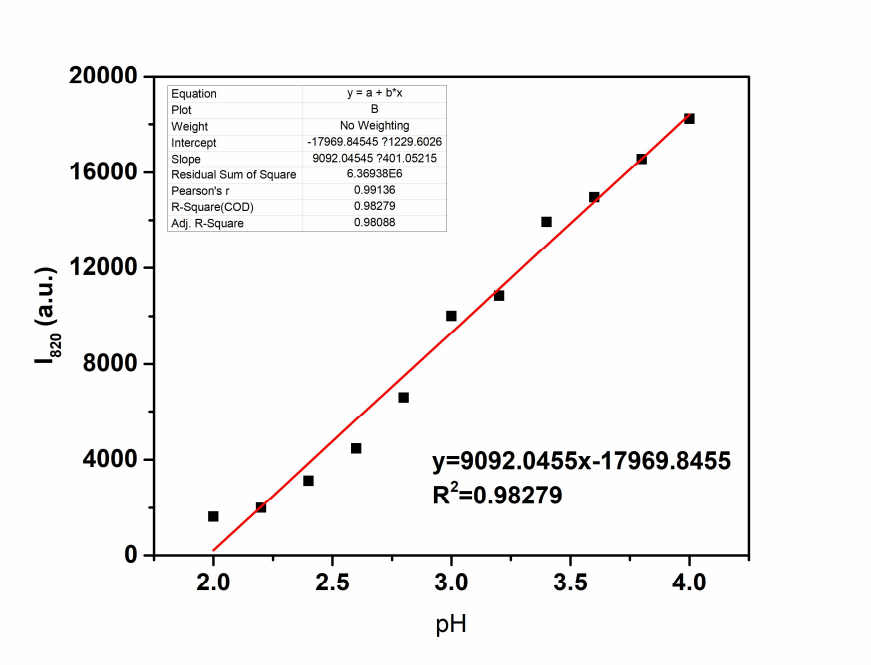

Fig. S8 The linear relationship between fluorescence intensity of **Hcy-pH** (10 μM) at 820 nm and different pH values. $\lambda_{ex}/\lambda_{em}$= 580 /820 nm, slit width (ex/em) = 10/10 nm.

5. Verification of sensing mechanism of Hcy-pH for proton

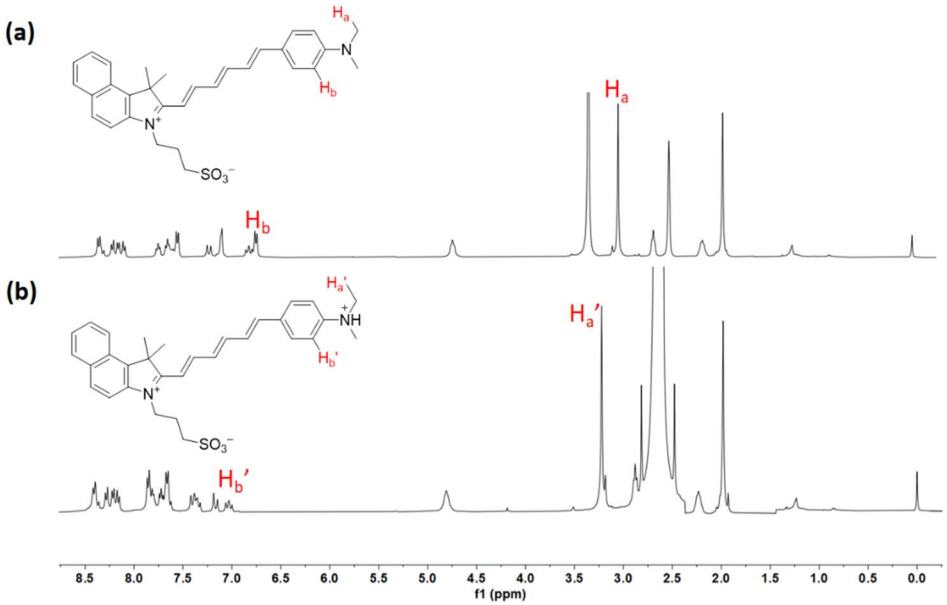

Figure S9 ^{1}H NMR spectra of **Hcy-pH** before (a) and after (b) the addition of methanesulfonic acid (400 MHz, DMSO-d_6).

6. Determination of quantum yield of Hcy-pH

The UV absorption spectra of **Hcy-pH** (0.25, 0.5, 1.5 μM) and Rhodamine B (2.0, 4.0, 6.0 μM) were measured using a UV spectrophotometer and repeated three times. When the concentrations of **Hcy-pH** and Rhodamine B were set at 0.25 μM and 2 μM, respectively, the isosbestic point between their absorption spectra was at 558 nm. Then, the fluorescence intensities of **Hcy-pH** (0.25 μM) and Rhodamine B (2.0 μM) were measured with 558 nm as the excitation wavelength and the fluorescence integral areas were calculated. The procedures were repeated three times. Finally, the fluorescence quantum yield could be calculated according to the formula $\Phi_X = \Phi_S \cdot \frac{A_S}{A_X} \cdot \frac{E_X}{E_S} \cdot \frac{n_X^2}{n_S^2}$, where X is the sample to be tested, S is the reference substance, Φ is the fluorescence quantum yield, E is the integrated fluorescence intensity, A is the solution absorbance, and n is the solution refractive index. The mean fluorescence quantum yield of **Hcy-pH** was determined to be 0.112.

Table S2 Fluorescence quantum yields of probe **Hcy-pH**.

	A(Abs)/E	A(Abs)/E	A(Abs)/E
Hcy-pH	0.064/243610.5	0.053/249502.95	0.054/235749.35
Rhodamine B	0.051/1626255.75	0.065/1677378.15	0.064/1633205.75
Fluorescent quantum yield	0.111	0.113	0.113

7. Cell viabilities of Hela cells after incubation with Hcy-pH

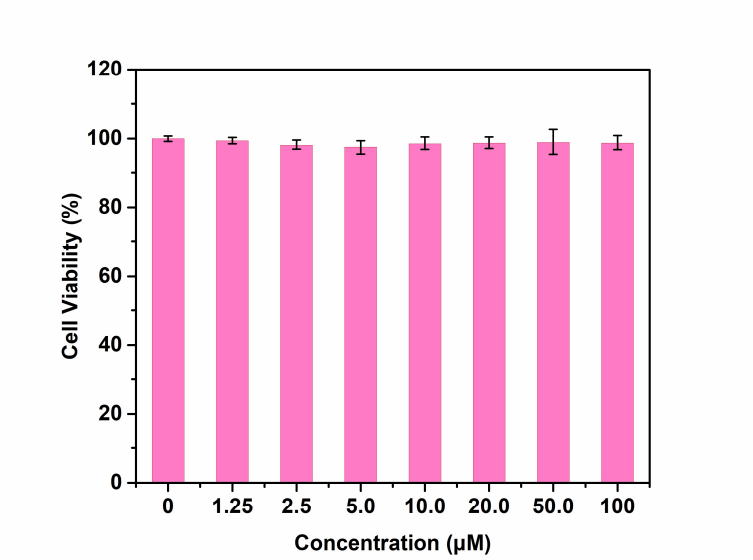

Fig. S10 Cell viabilities of Hela cells after incubation with different concentrations of **Hcy-pH** (0-100 μM) for 24 h.