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Electronic Supplementary Information (ESI)

A Cell-Surface-Anchored Ratiometric I-Motif Sensor for Extracellular pH Detection

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Experimental Section

1. Materials and Reagents

The sequence of probe (1) and probe (2) were adopted from Literature [1]. They were purchased from TAKARA Biotechnology Inc. (Dalian, China) and purified by reverse-phase high performance liquid chromatography (HPLC). Sulfonated biotinyl-N-hydroxy-succinimide (NHS-Biotin) and streptavidin were purchased from Sangon Biotechnology Co., Ltd (Shanghai, China).

Phosphate Buffer (PB): 100 mM Na_2HPO_4, 100 mM NaH_2PO_4. The pH depends on the ratio. Phosphate Buffered Saline (PBS): 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 2 mM KH_2PO_4, pH 7.4. All aqueous solutions used in the experiments were prepared using ultrapure water(≥18MΩ, Milli-Q water purification system, Millipore).

2. Instrumentation

The fluorescent spectra were measured using a Hitachi F-7000 fluorescence spectrometer (Tokyo, Japan). Circular dichroism (CD) spectra were using a Biologic MOS-500 CD spectrophotometer (Claix, France). The confocal microscope measurements were obtained using an Olympus laser-scanning confocal microscope (Tokyo, Japan). The Flow cytometry analysis was gained from Gallios machine (Beckman Coulter, USA). Cell incubation was done using a humidified HF90 CO2 incubator (Shanghai Lishen Scientific Equipment Co. Ltd.). All pH measurements of buffer were performed with Thermo Scientific Orion 3 Star pH-meter (Waltham, MA, USA). Centrifuge was performed with Beckman Coulter Allegra 25R centrifuge (Brea, CA, USA).

3. Fluorescence Experiments in vitro

The pH probes were diluted to a concentration of 50 nM in PB and treated with different pH buffer (pH 5.0-7.0). The fluorescence spectra were recorded on an F-7000 fluorescence spectrometer exciting at 488 nm and measuring emission from 510 to 660 nm in 1 nm increments. Both excitation and emission slits were set at 10 nm.

4. Cell culture

HeLa cells (human cervical cancer cell line), L02 cells (human hepatocyte cell line), SMMC-7721 cells (human hepatocyte cell line) and CCRF-CEM cells (human acute lymphatic leukemia cell line) were grown in RPMI 1640 medium supplemented with 10% inactivated fetal bovine serum, 100 U/ml 1% penicillin and streptomycin solution. All cells were cultured in a humidified CO_2 incubator containing 5% CO_2 at 37°C.

5. Engineering pH Probes onto the Cell Surface

HeLa cells were incubated with NHS-Biotin (1 mM in PBS, pH 7.4) at room temperature for 30 min. After washing three times by PBS, streptavidin solution (50 μg/mL in PBS, pH 7.4) was then used to treat the cells for 20 min. Finally, 125 nM biotin-modified probe solution (two DNA strands were first annealed in PBS at 95 °C for

2 min and slowly cooled to room temperature) was added, incubated for 1 h on ice. The cells were then washed to remove the unbound DNA probes and subsequently used for experimentation.

6. Confocal Fluorescence Microscopy Imaging

To study the location of biotin-modified probe on the cell membrane, HeLa cells were treated with streptavidin for 20 min after biotinylating the cell surface and then incubated with biotin-modified DNA probes for 1 h. Subsequently, the cells were washed to remove excess probes by PBS and stained with 30 nM DAPI for 10 min. Finally, the cells were washed three times, resuspensed in PBS and imaged on a laser-scanning confocal microscope.

For extracellular pH measurement, cells were biotinylated with NHS-Biotin followed by conjugation with streptavidin as described above. After the incubation with DNA probes, the cells were washed with PBS and resuspensed in different extracellular pH buffers for confocal imaging. The fluorescence images and ratio fluorescence images were presented after processing by software Image Proplus 6.0.

7. Flow Cytometric Assay

HeLa cells were incubated with NHS-Biotin at room temperature for 30 min. After washing three times by PBS, streptavidin solution was then used to treat the cells for 20 min. Subsequently, biotin-modified DNA probes was added, incubated for 1 h on ice. The cells were then washed to remove the unbound DNA probes. After treatment, cells were detached from culture dishes using Trypsin-EDTA Solution. The solution containing treated cells was centrifuged (2000 rpm, 4 min) and resuspended in PBS three times. Flow cytometric assay was performed using Beckman Coulter Gallios machine.

References

[1] S. Modi, C. Nizak, S. Surana, S. Halder, and Y. Krishnan, *Nat. Nanotechnol*, 2013, **8**, 459.

Supporting Figures

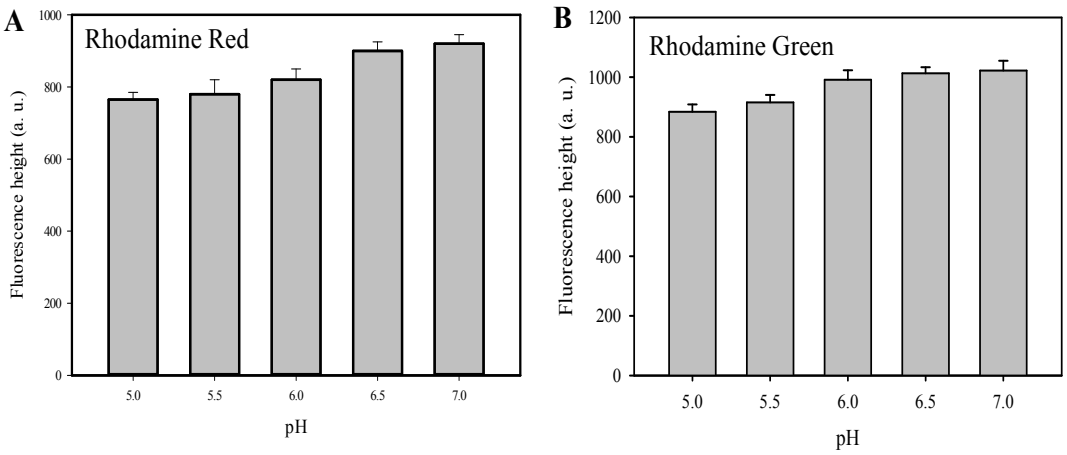

Figure S1. Stability of Rhodamine Red (A) and Rhodamine Green (B) under various pH conditions (pH 5-7).

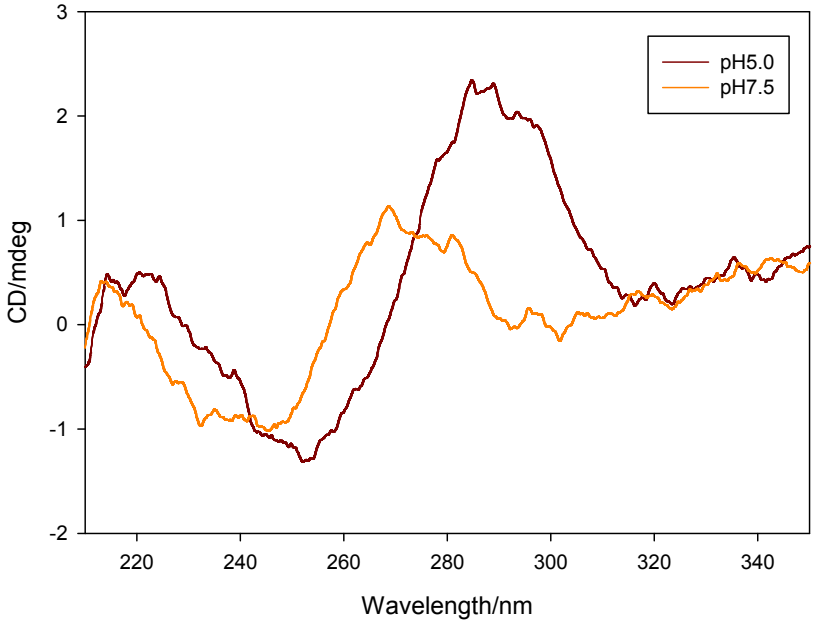

Figure S2. CD spectra of the i-motif sequence in pH 5.0 and pH 7.5 solutions.

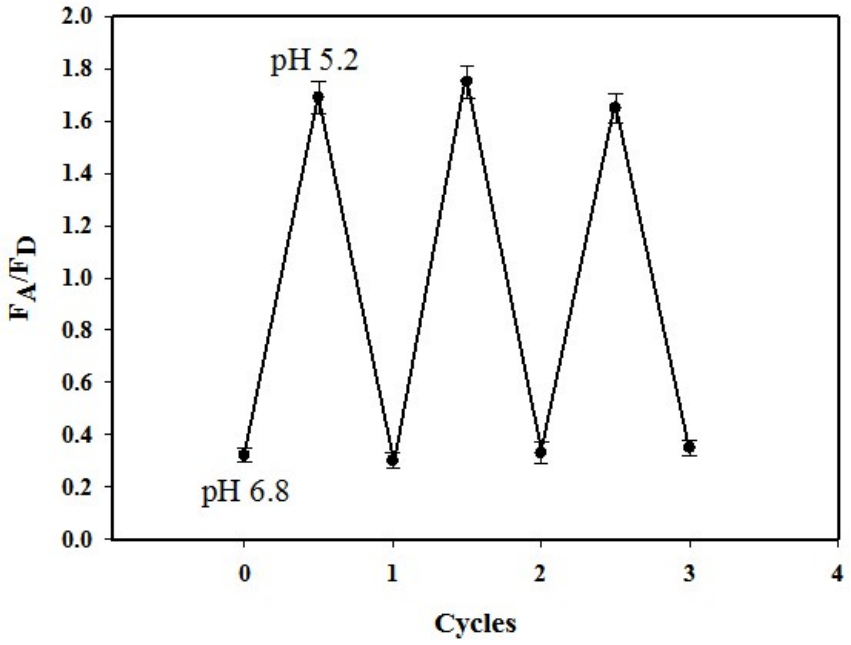

Figure S3. The reversible change of fluorescence intensity ratio in response to extracellular pHs of 5.2 and 6.8.

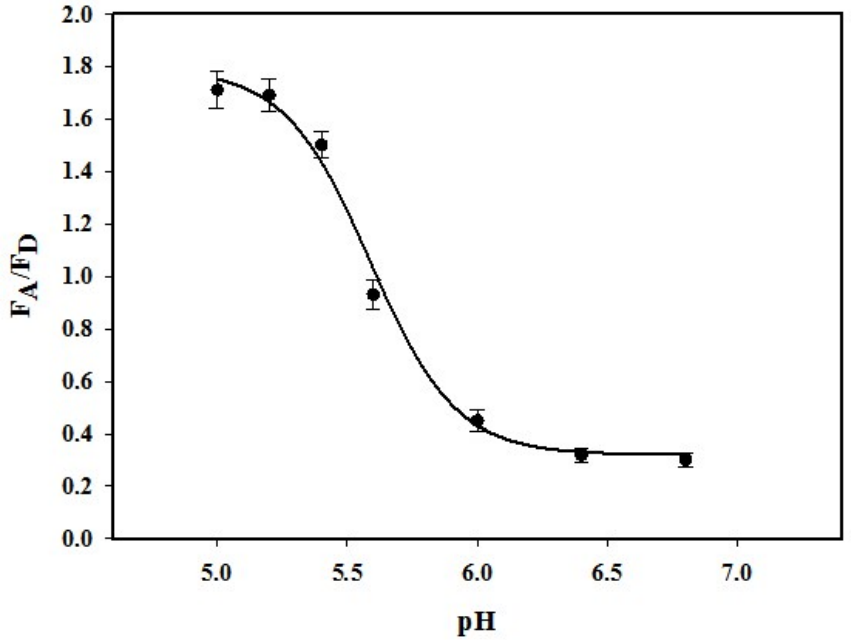

Figure S4. Plot of emission intensity ratio (A/D) of HeLa cells modified with i-motif probes vs extracellular pH.