Supplementary Information

Programming cell communications with pH-responsive DNA nanodevices

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Reagents and materials

All DNA oligonucleotides were synthesized and purified by Sangon Biotech Co., Ltd. (Shanghai, China). Cell lines Ramos was purchased from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). CellTracker™ Dyes (Green, Deep Red and Violet) were obtained from Thermo Fisher Scientific Inc (Waltham, MA, USA). All other chemical reagents were of analytical grade and purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). All solutions were prepared using ultrapure water obtained through a Millipore Milli-Q water purification system (Billerica, MA, USA), with an electric resistance of >18.2 MΩ.

Instrumentation

The fluorescence measurements were carried out at room temperature using Fluorescence Spectrometer FS900 (Edinburgh Instruments, UK). All fluorescence images were acquired using an oil dipping objective (63×) on a Leica SP8 confocal microscope (Leica; Germany). Stained cells and COCs were examined and sorted by FACS flow cytometry Image Stream^x Mark II (Merck KGaA, Germany).

Fluorescence Measurements

For in vitro fluorescence experiments, 20 nM **DTN-BHQ-AL488** was incubated in 100 μL PBS buffer at different pH at room temperature for 30 min. The fluorescence spectra were recorded at room temperature using Fluorescence Spectrometer FS900 (Edinburgh Instruments, UK) and collected emission spectra from 500 nm to 600 nm with a 493 nm excitation wavelength. All the excitation and emission slits were set at 5.0 nm. Dwell time was set at 0.1 S.

Cell culture and fluorescence imaging

Ramos cell was cultured in RPIM 1640 medium supplemented with 10% fetal bovine serum, penicillin/streptomycin (100 units/mL) and L glutamine (2 mM) at 37 °C in humidified environment containing 5% CO_2.

For cytosolic staining, CellTrackers were first dissolved in DMSO to 1 mM (1000×) and further diluted to a final working concentration of 1 μM in medium. Cells were washed twice (1200 rpm, 3 min) in PBS buffer to remove any serum and then incubated in stain solution for 30 min at room temperature. Labeled cells were washed twice in PBS buffer and re-suspended in cell culture medium.

For DNA modification on cell membrane, 5×10^6 Ramos cells were washed with 1 mL 1×PBS buffer three times (1200 rpm, 3 min) to remove any serum from the culture medium. The cells were then exposed to solutions of **GGT-chol** (500 nM, 100 μL) for 30 min at room temperature. After isolation via centrifugation (1200 rpm, 3 min) for three times, the cells were resuspended to 100 μL PBS buffer.

For the reversibility studying in Ramos cells, Ramos cells were incubated with 500 nM **GGT-chol** in 100 μL 1×PBS buffer at room temperature for 30 min, washed (1200 rpm, 3 min) twice with 1 mL 1×PBS buffer and resuspended to 100 μL 1×PBS buffer. Then, 2 μM **ACC-DTN-BHQ-AL488** were incubated with cells at room temperature for 30 min, washed twice with 1 mL 1×PBS buffer at pH 7.5 and resuspended to 100 μL 1×PBS buffer at pH 7.5 for imaging. Next, washed twice with 1 mL 1×PBS buffer at pH 6.5 and resuspended to 100 μL 1×PBS buffer at pH 6.5 for another 30 min and imaging. The incubating buffer was replaced at pH 7.5 for 30 min again and imaging.

For the responsiveness studying in Ramos cells, Ramos cells were incubated with 500 nM **GGT-chol** in 100 μL 1×PBS buffer at room temperature for 30 min, washed (1200 rpm, 3 min) twice with 1 mL 1×PBS buffer and resuspended to 100 μL 1×PBS buffer. Then, 2 μM **ACC-DTN-BHQ-AL488** were incubated with cells at room temperature for 30 min, washed twice with 1 mL 1×PBS buffer at various pH values (pH 4.5, 5.1, 5.5, 6.1, 6.5, 6.8, 7.1, 7.5, 8.0, 8.5 and 9.4) and resuspended to 100 μL 1×PBS buffer at various pH values for detecting.

For assembly of cell organization, Ramos cells (250000 cells/mL, 1 mL) were seeded in 24-well plate and grown overnight. After cytosolic staining, cells were modified with **GGT-chol** and **chol-GAA.** The DNA modified cells were

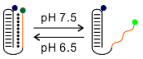
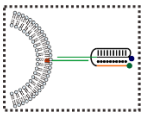
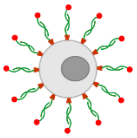
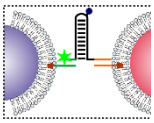
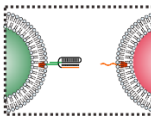
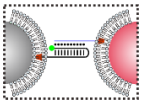
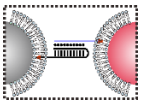
mixed together at a 1:1 ratio in 100 µL 1×PBS buffer，cells were previously washed twice in 1×PBS solutions at different pH values (pH 6.5 and 7.5). Corresponding complementary **ACC-DTN** (final concentration 2 µM) were added to the mixtures, after 30 min incubation at room temperature, the assembled patterns were analyzed by confocal or flow cytometry as required.

All fluorescence images were acquired using an oil dipping objective (63×, 1.25 NA) on a Leica SP8 confocal microscope. Wavelength sets were 488 nm excitation (Ex)/500-600 nm emission (Em) for Alexa488 and CellTracker Green, 405 nm Ex/440-490 nm Em for CellTracker Violet, and 633 nm Ex/650-690 nm Em for CY5 and CellTracker Deep Red.

Fluorescence-activated cell sorting (FACS) analysis

The setup of excitation wavelengths for FACS analysis and cell sorting followed the manuals of the fluorescent dyes. The detection of cellular fluorescence signal was performed on Image Stream^{x} Mark II by counting 5000 events, using the 488nm channel for Alexa488 and CellTracker Green, 405 nm channel for CellTracker Violet, and 633 nm channel for CY5 and CellTracker Deep Red. The data were calculated from three independent experiments.

Table S1. DNA sequence used in the experiment

No.	Sequences Name	Sequences (5′ – 3′)	Scheme	Scenarios
1	DTN-BHQ-AL488	AAGGAAGAAGTTT(BHQ-1) ACTTCTTCCTTCTTTGTTCCTTCTTC-Alexa Flour488		Figure S1 
2	ACC-DTN-BHQ-AL488	ACCACCACCACCACCACCAAAAGGAAGAAGTTT(BHQ-1)ACTTCTTCCTT CTTTGTTCCTTCTTC-Alexa Flour488		Figure 1a, Figure S3 and S4 
3	GGT-chol	GGTGGTGGTGGTGGTGGTTT- cholesterol		
4	ACC-CY5	ACCACCACCACCACCACCAA-cy5		Figure S2 
5	ACC-DTN-BHQ-AL488	ACCACCACCACCACCACCAA AAGGAAGAAGTTT(BHQ-1)ACTTCTTCCTTCTTTGTTCCTTCTTC-Alexa Flour488		Figure S5 
6	GGT-chol	GGTGGTGGTGGTGGTGGTTT- cholesterol		
7	chol-GAA	Cholesterol-TTGAAGAAGGAA		
8	chol-GAA	Cholesterol-TTGAAGAAGGAA		Figure 2a-c, Figure S6a, S6b and S7
9	ACC-DTN	ACCACCACCACCACCACCAAAAGGAAGAAGTTTACTTCTTCCTTCTT TGTTCCTTCTTC		
10	GGT-chol	GGTGGTGGTGGTGGTGGTTT- cholesterol		
11	chol-tri488	Cholesterol-TTAAGGAAGAAGTTTACTTCTTCCTT-Alexa Flour488		Figure 2d-f, Figure S6c, S6d and S8
12	AAG-chol	AAGGA AGAAG TT -cholesterol		
13	chol-tri	Cholesterol-TTAAGGAAGAAGTTTACTTCTTCCTT		
14	AAG-chol	AAGGA AGAAG TT -cholesterol		

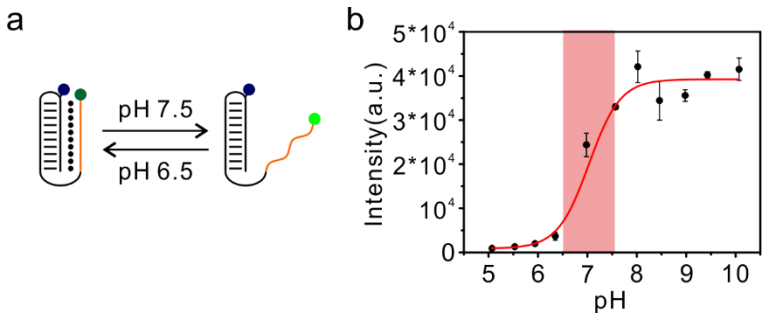


Figure S1. Triplex DNA nanoswitch can be triggered over a specifically defined pH window. Triplex – to – duplex transition is monitored through a pH – insensitive fluorophore (Alexa Fluor 488, AL488) inserted at the 5' – end and a quencher (Black – Hole Quencher 1, BHQ-1) internally located in the switch. The intensity increasing occurred over the pH range of 6.5 to 7.5. Error bars represent standard deviations from three independent experiments.

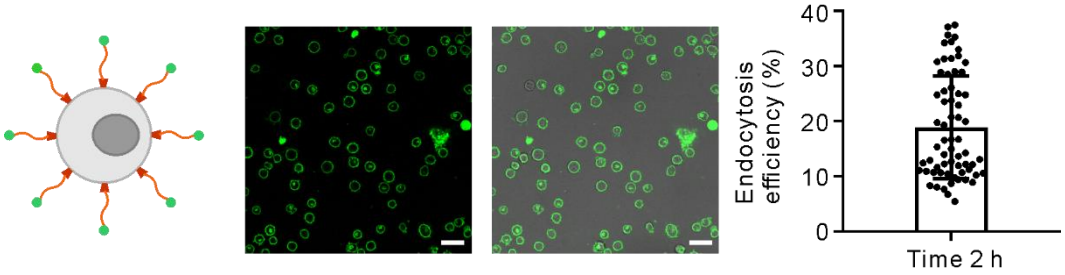


Figure S2. The confocal imaging results indicated cholesterol-modified DNA was slowly internalized into Ramos cells. on cell membrane. At 2h after incubation, 18.9 ± 8.0% of fluorescence intensity was observed in cells, taking the fluorescence intensity in cytoplasm and on cell membrane together as 100%. Scale bar 25 μm. N=65.

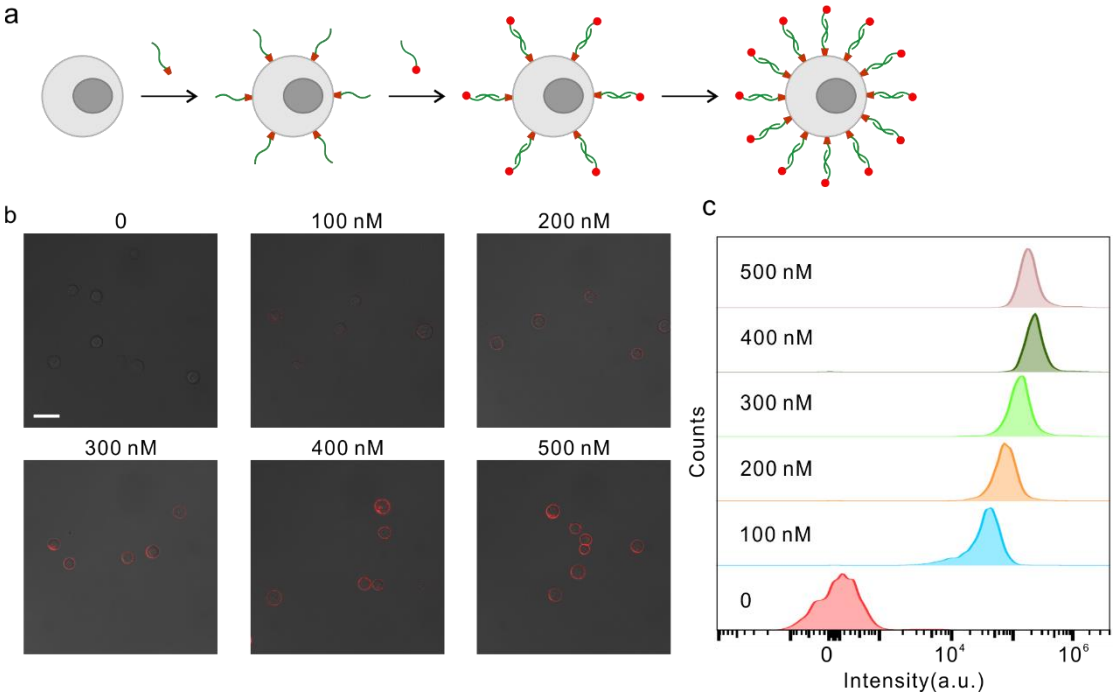


Figure S3. (a) Schematic illustration and (b) confocal images of DNA modifications on cell membrance. The CY5-labeled DNA strands were hybridized with the cholesterol modified ssDNA on cell surface for different DNA concentrations. The inset scale bar was 25 μm; (c) FACS analysis of cell fluorescence upon incubation of different estimated DNA densities on the cell concentrations.

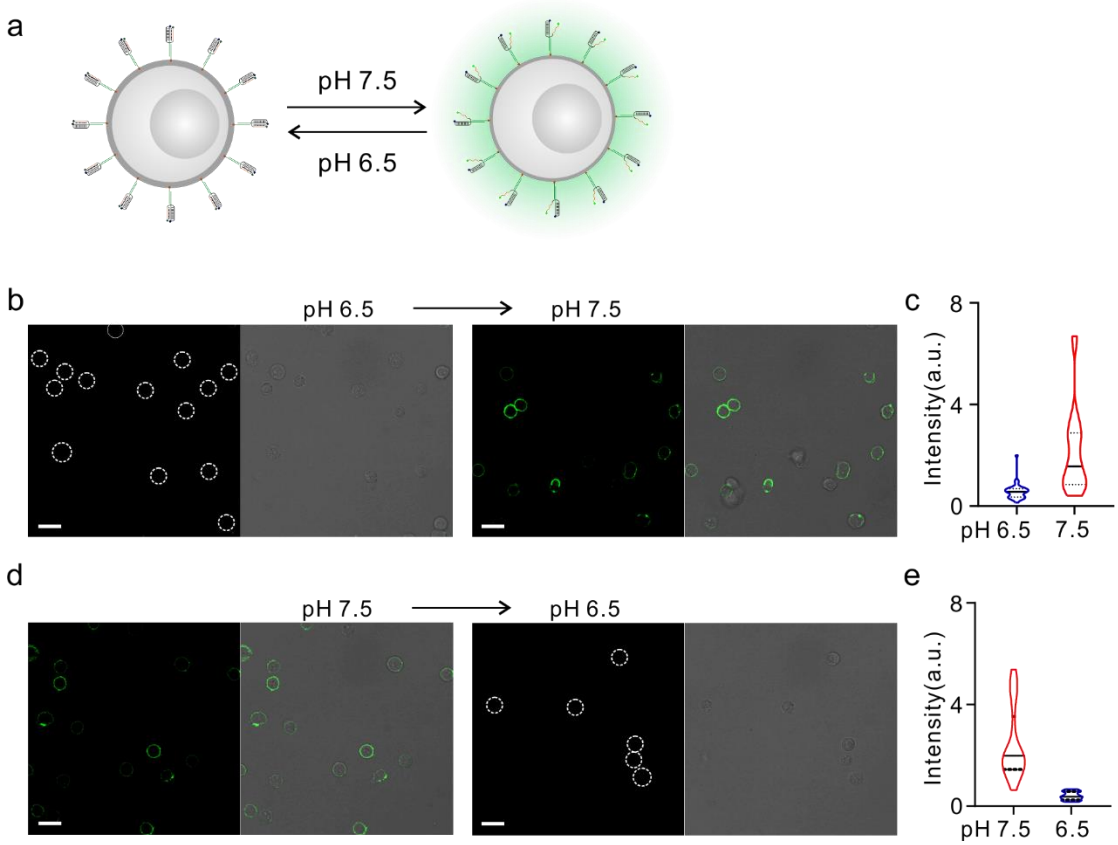


Figure S4. (a) Schematic illustration and (b) Confocal images of living Ramos cells modified with DTNs at pH 6.5 to 7.5. The inset scale bar was 25 μm and the white dash circles indicated the Ramos cells; (c) Quantification analysis of AL488 fluorescence intensity from confocal images, over 15 cells were measured with ImageJ software for each sample group; (d) Confocal images of living Ramos cells modified with DTNs at pH 7.5 to 6.5. The inset scale bar was 25 μm and the white dash circles indicated the Ramos cells; (e) Quantification analysis of AL488 fluorescence intensity from confocal images, over 20 cells were measured with ImageJ software for each sample group.

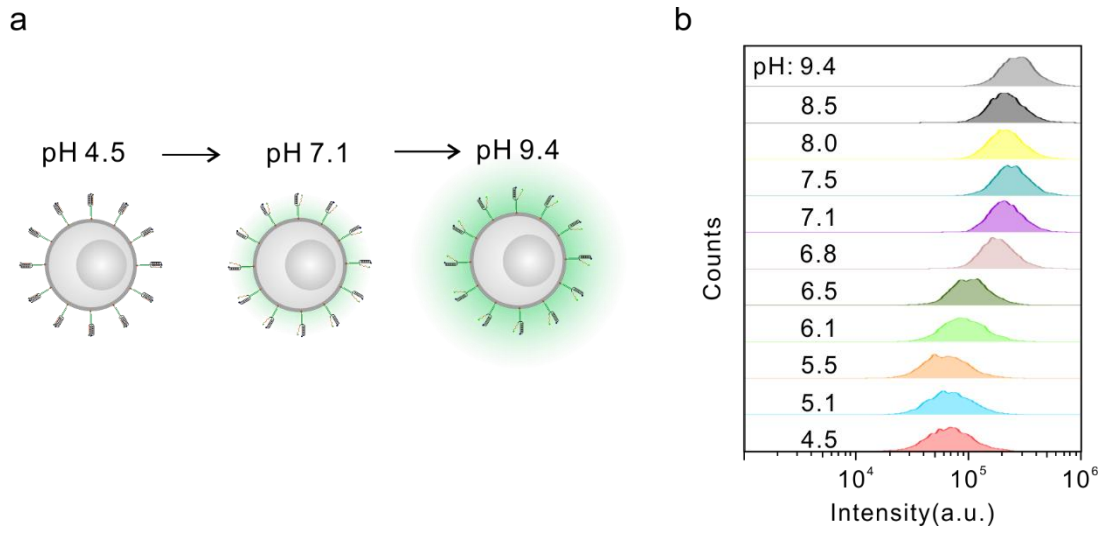


Figure S5. (a) Schematic illustration and (b) FACS analysis of the mean fluorescence intensity of Al488 on Ramos cells at different pH values.

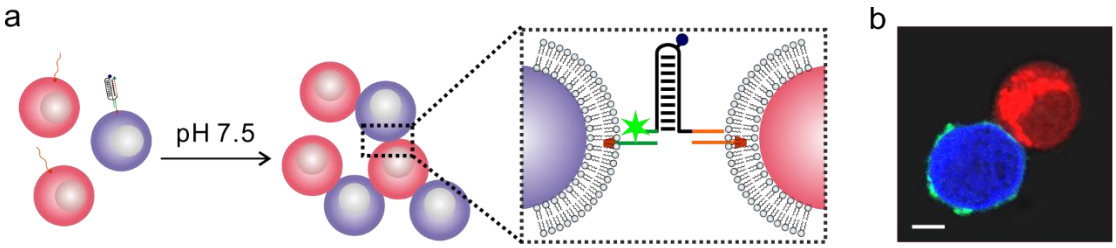


Figure S6. (a) Scheme of triplex DNA based organization of cells in physiological condition (pH 7.5). Two group sets of cells were stained with CellTracker violet and deep red respectively, while AL488 (green) and BHQ-1 were simultaneously recruited in DTNs; (b) Confocal image of cell assembly induced by pH trigger. The inset scale bar was 5 μm.

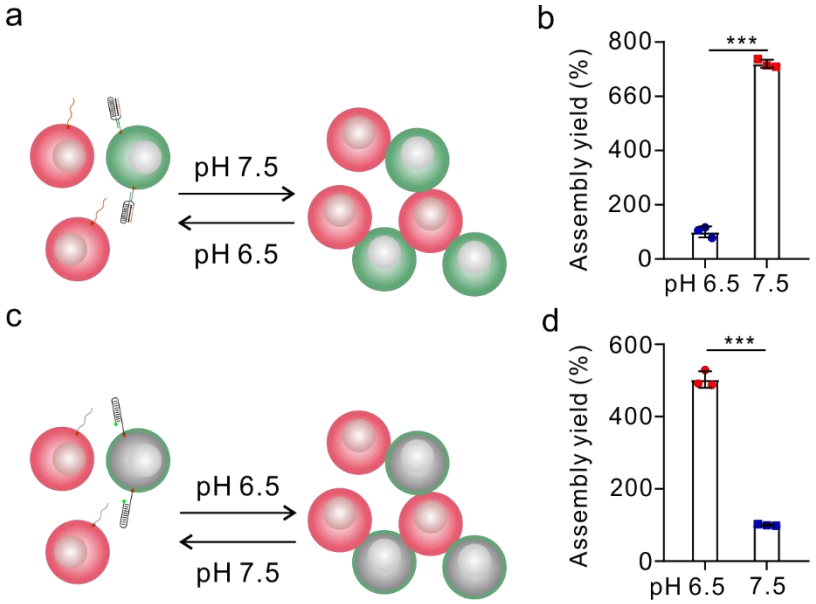


Figure S7. (a, c) Schematic illustration and the assembly yield of DTN-directed cell organization in both physiological condition (pH 7.5) (b) and acidic condition (pH 6.5); (d). The assembly yield was calculated from the FACS data in Figure 2d & 2e. (***P < 0.001, Student-t test.)

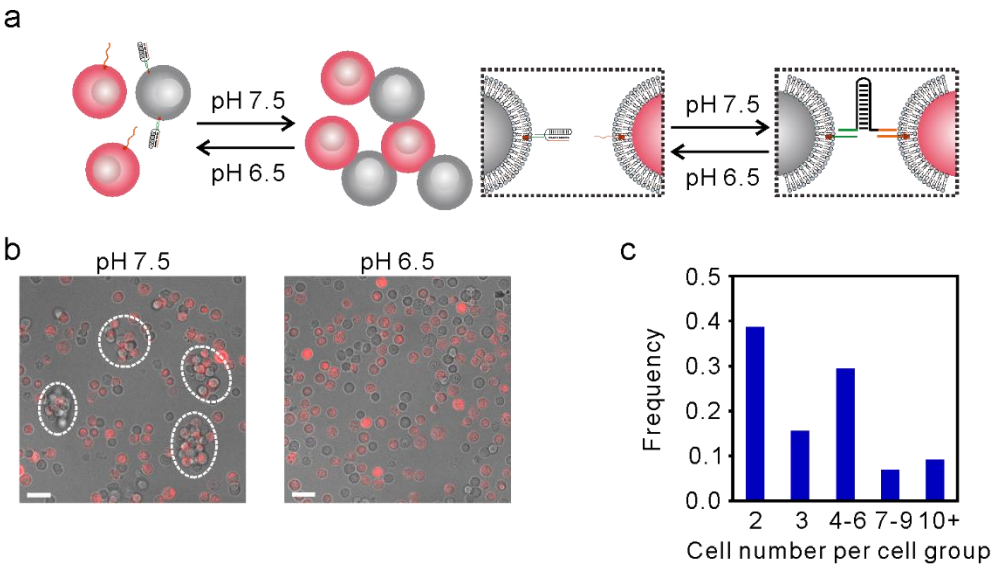


Figure S8. (a) Schematic illustration and (b) Confocal images of cell assembly in physiological condition (pH 7.5). The inset scale bar was 25 μm; (c) Cell number distributions of each group in alkaline condition. Over 100 cell groups were measured.

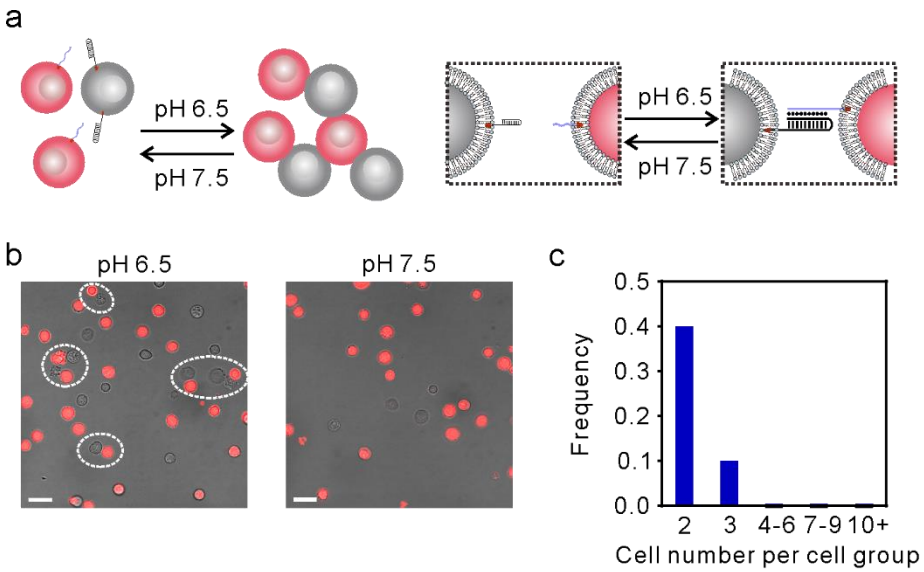


Figure S9. (a) Schematic illustration and (b) Confocal images of cell assembly in acidic condition (pH 6.5). The inset scale bar was 25 μm; (c) Cell number distributions of each group in acidic condition. Over 15 cell groups were measured.