Local pH Tracking in Living Cells

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Synthesis of FMSNs(-)

Ratiometric Fluorescence mesoporous silica nanoparticles (FMSNs) were prepared by conjugating both FITC and RITC to the MSNs through ATPMS while the growth of MSNs. Initially, 0.29 g of CTAB was dissolved in 150 g of 0.51 M ammonium hydroxide water solution, and then 2.5 mL of 0.41 M TEOS ethanol solution was added. The mixture was sealed in flask with parafilm and heated to 40 ^{o}C under continuous stirring. After 4-hour stirring, the parafilm on the flask was removed, then 8.1 mL of APTMS-conjugated FITC ethanol solution and 3.4 mL of 0.89 M of TEOS ethanol solution were sequentially added into the mixture. After stirring for one hour, 0.54 mL of APTMS-conjugated RITC ethanol solution was then added, and then the mixture was stirred for another hour. After the mixture was aged at 40 ^{o}C without stirring for 24 hours, the pH value of solution was altered to 10 by adding ammonium, and then 2 mL THPMP aqueous solution (0.5 mL in 2 mL water) was added. The mixture was sealed in flask again and heated to 40 ^{o}C under continuous stirring for 2 hours. The resulting product was washed by two routines to remove surfactant

template and unwanted physical absorbed chemicals. Routine 1: The resulting product (FMSN(-)) was then collected and dispersed in 25 mL of ethanol with sonication and then centrifuged to collect the precipitated product. Routine 2: the precipitated product was dispersed in the ammounium nitrate/ ethanol solution (6 g/ 50 mL) and stirred at 60 ^{o}C for one hour. First, perform Routine 1 for four times, and then perform Routine 2 once. Second, perform Routine 1 for three times, and then perform Routine 2 once but using half amount of ammounium nitrate (3g/ 50 mL) Finally, repeat Routine 1 for three times, and the resulting FMSNs(-) were stored in 99.5% ethanol in the dark.

Culture Cells.

HeLa cells, human cervical carcinoma cells, were maintained in Dulbecco's modified Eagles medium (DMEM; GIBCO), 10% fetal ovine serum (FBS; GIBCO) and 1% antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin; GIBCO). The HeLa cells were incubated at 37 ^{o}C under a humidified atmosphere of 95% air and 5% CO_2. The cells were grown in T175 flask with a sealed cap. When adherent cells reached ~ 60% to 70% confluence, the growth medium was removed and the cells were washed twice with PBS and then 2 mL 0.25% trypsin-EDTA was added. The flask was placed in the incubator at 37 °C under a humidified atmosphere of 95% air and 5% CO_2 for 5 min. The cells were

then dislodged from the flask surface and were mixed with fresh growth medium in a 15 mL centrifuge tube. To remove the excess of trypsin, the solution was centrifuged with 1200 rpm for 3 min. The supernatant was discarded and the pellet was resuspended in growth medium and placed in a clean T175 flask. For imaging, HeLa cells were cultured on 18 mm diameter glass coverslips one day prior to performing imaging experiments. Cells on coverslips were cultured in growth medium in 6-well Nunc multidishes at 37 °C in a 95% air and 5% CO_2 atmosphere.

Incubation of HeLa cells with FMSNs(+)

The HeLa cells cultured on 18 mm diameter glass coverslips were washed three times with PBS, then 1 mL DMEM (without serum and phenol red) mixed with 100 μg FMSNs(+) and 10 μL/mL FM4-64 dye (dissolved in Hank's Balanced Salt Solution (HBSS) first) was added to each well of 6-well Nunc multidishes and incubated at 37 °C in a 95% air and 5% CO_2 environment for 4 h. The supernatants were removed and cells were washed three times with HBSS, and kept in HBSS for observation by confocal microscopy (TCS SP5, Leica).

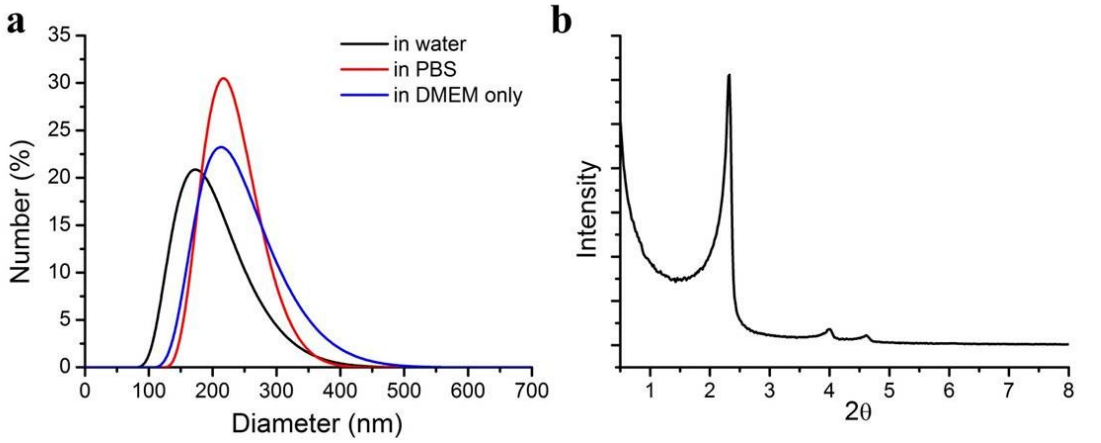


Figure S 1 (a) Particle size distribution of FMSN(+). The hydrated size of FMSN(+) in different solutions is determined by dynamic light scattering method. (b) The XRD pattern of FMSN(+).

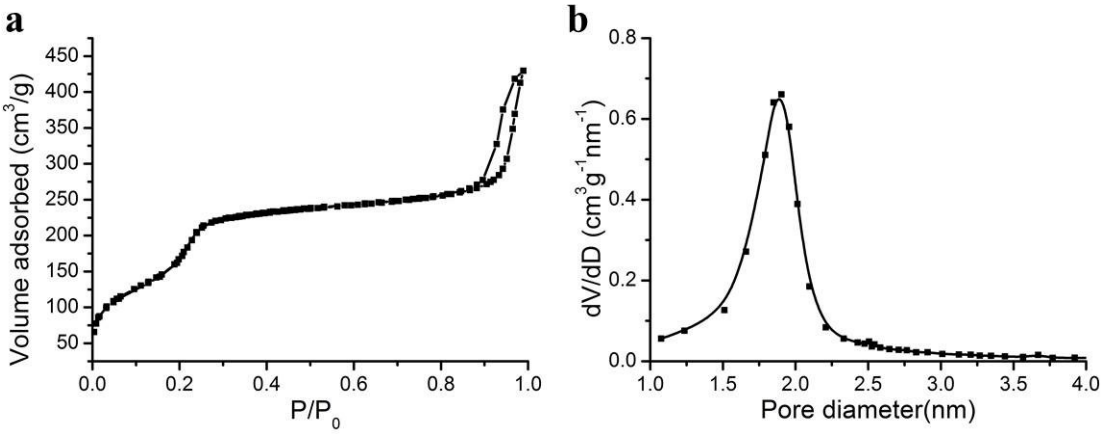


Figure S 2 Nitrogen adsorption-desorption isotherm (a) and corresponding pore size distribution plot (b) of FMSN(+). The pore size distribution plot is obtained from the analysis of the adsorption or desorption isotherms using BJH (Barrett–Joyner–Halenda) method.

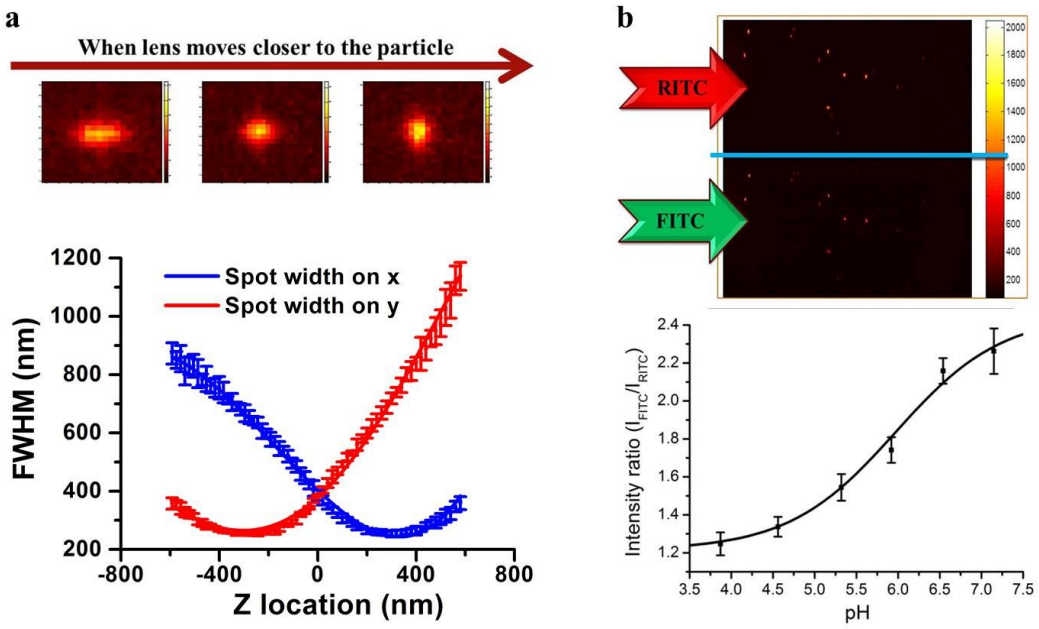
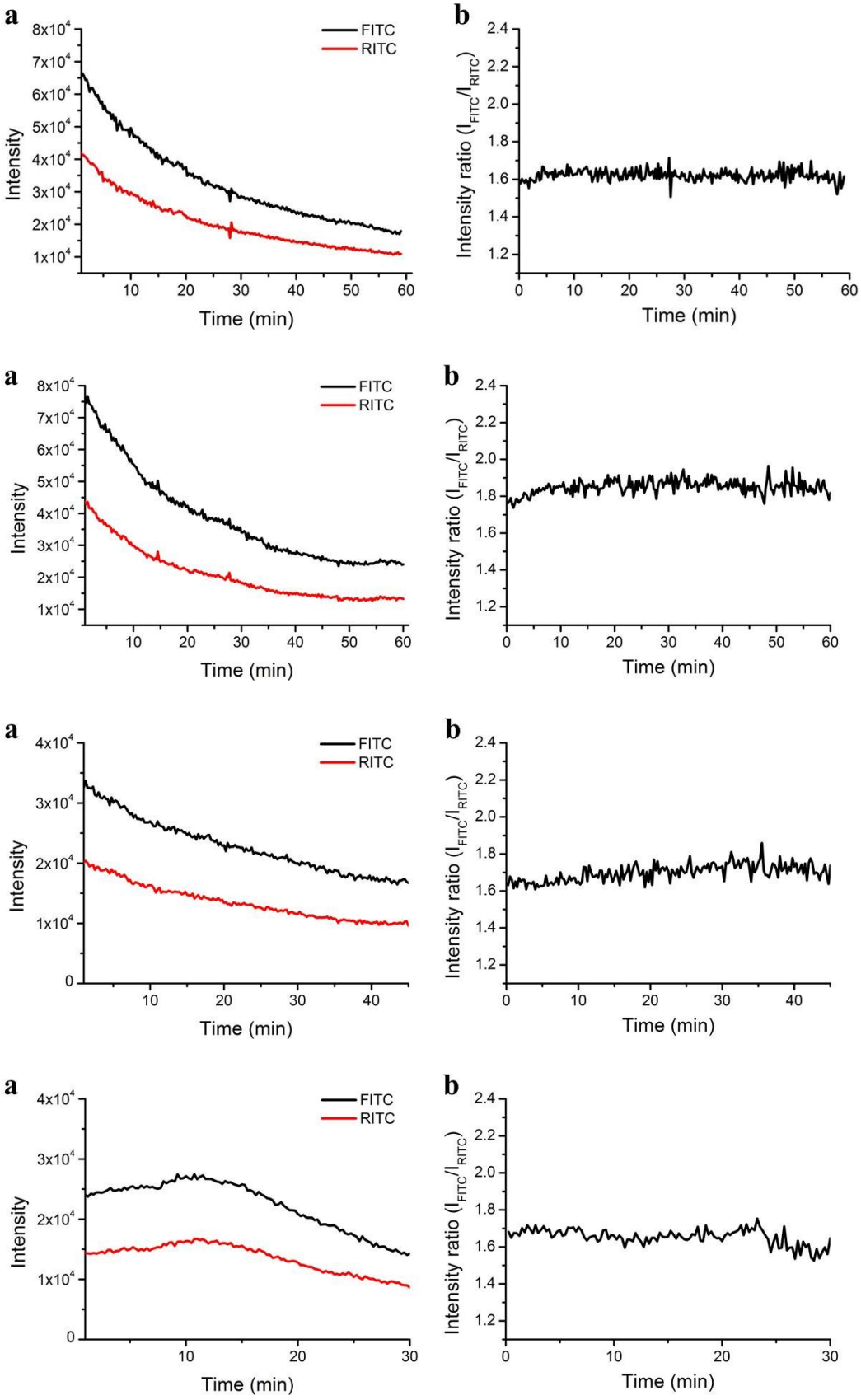


Figure S 3 Explanations of how calibration curves generated by wide-field based microscopy (curves are showed in Fig. 2b&c). (a) Z calibration curve is established by weak cylindrical lens in front of electron multiplying-CCD camera which creates astigmated fluoresence spots. Once the particle moves from position below the focal plane of cylindrical lens to the position above which, the shape of fluorescence spot is changed. (b) pH calibration curve. Due to the FRET system in optical setting, the emissions are seperated into RITC and FITC channels by dichroic lens and collected simultanouesly.


a
b
FITC
RITC
Intensity
Time (min)
Intensity ratio (I_{FITC}/I_{RITC})

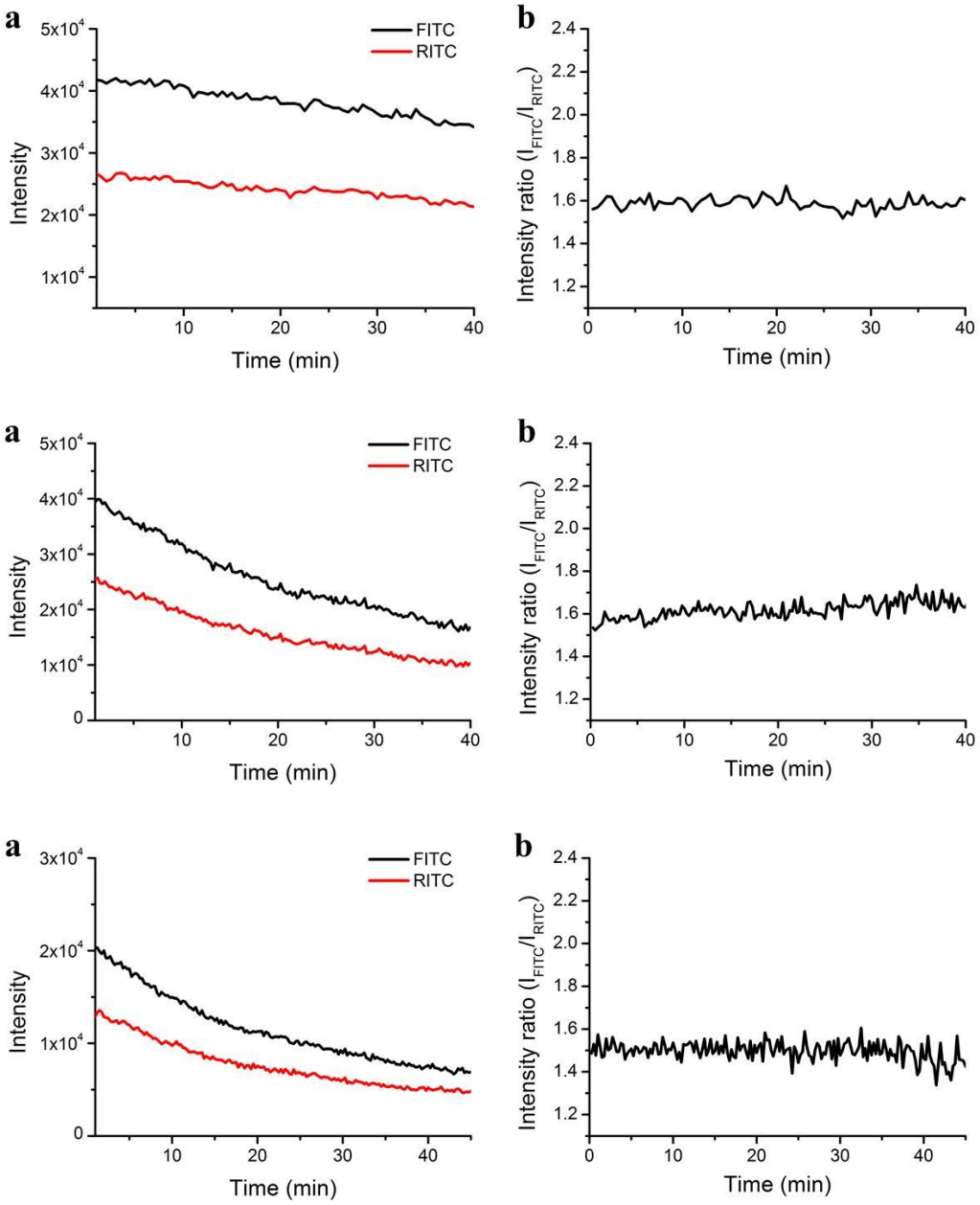


Figure S 4 Photo-stability of seven individual single FMSN(+)s. FMSNs(+) are immobilized on a cover glass for 1 h tracking (1 frame per 15 s, other settings are the same as single particle tracking experiment). (a) The intensity variation of both FITC and RITC within 1 h. (b) The corresponding intensity ratio, which remains the same in this duration.

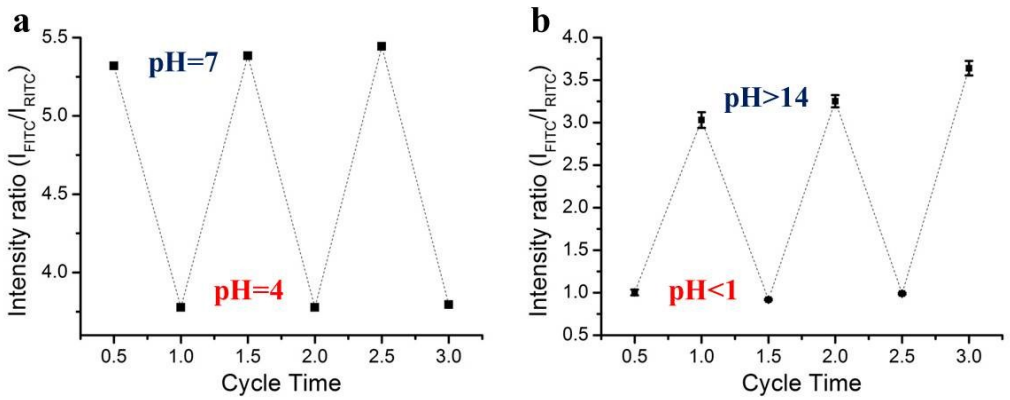


Figure S 5 (a) Reproducibility of FMSN(+). The pH of buffered solution is altered with NaOH and HCl aqueous solution and the test is demonstrated by fluorescence spectrophotometer. (b) Responsive rate of FMSN(+) using single particle tracking system. The pH of FMSN(+) contained solution is fastly changed between pH 1 and 14 by adding concentrate HCl and NaOH aqueous solution. Each cycle is measured three times within 20 s. Mean±SD is represented.

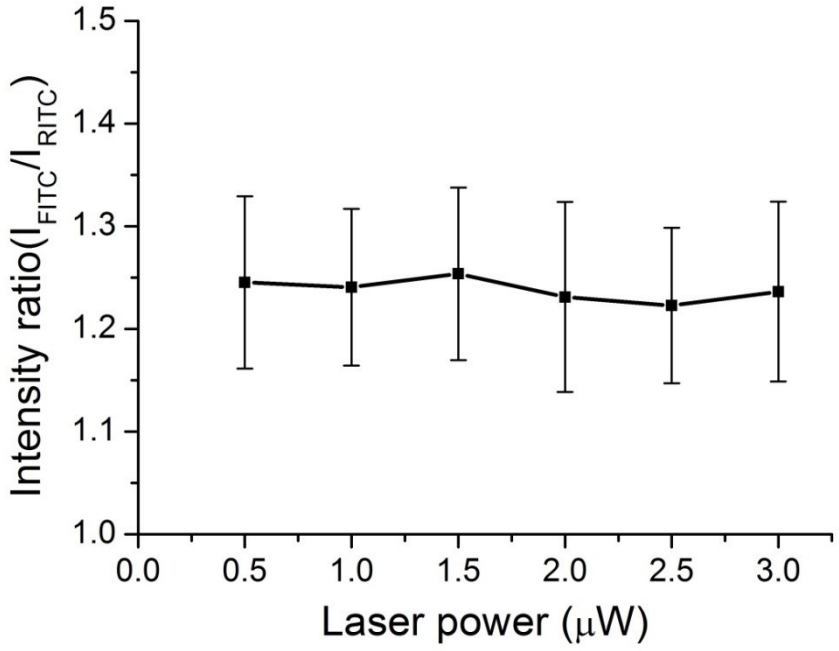


Figure S 6 Change of intensity ratio when FMSNs(+) are excited with different laser power. More than 30 particles in the same area are employed for calculation. Mean±SD is represented.

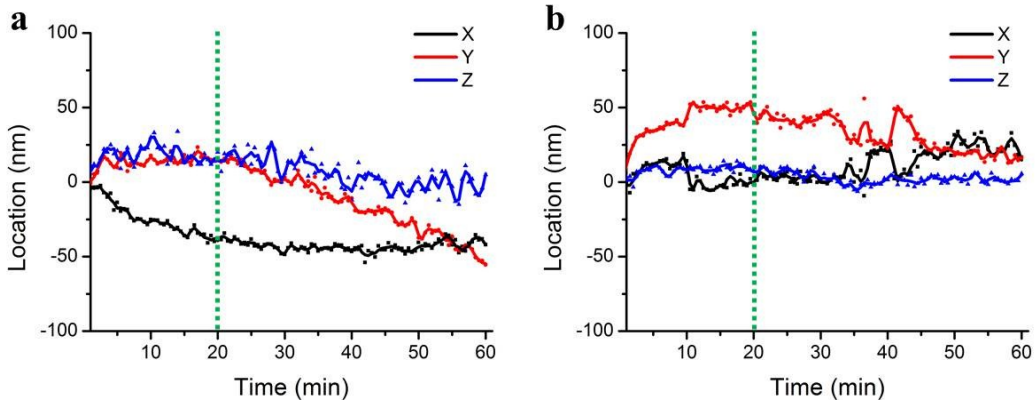


Figure S 7 (a) (b) X, Y and Z drift study. In these independent two cases, FMSNs(+) are immobilized on a cover glass for 1 h tracking (1 frame per 15 s, other settings are the same as single particle tracking experiment). The green dotted line indicates the lasting time of our single particle tracking experiments in HeLa cell.

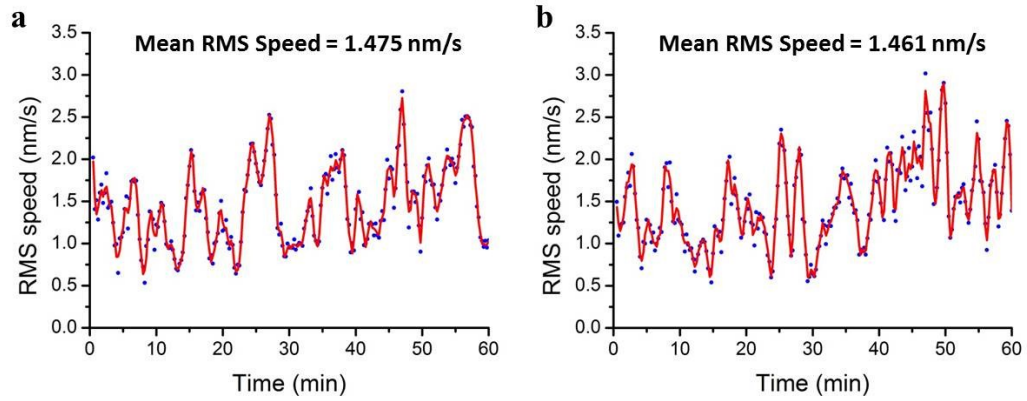


Figure S 8 The corresponding root mean square plots of Figure S7.

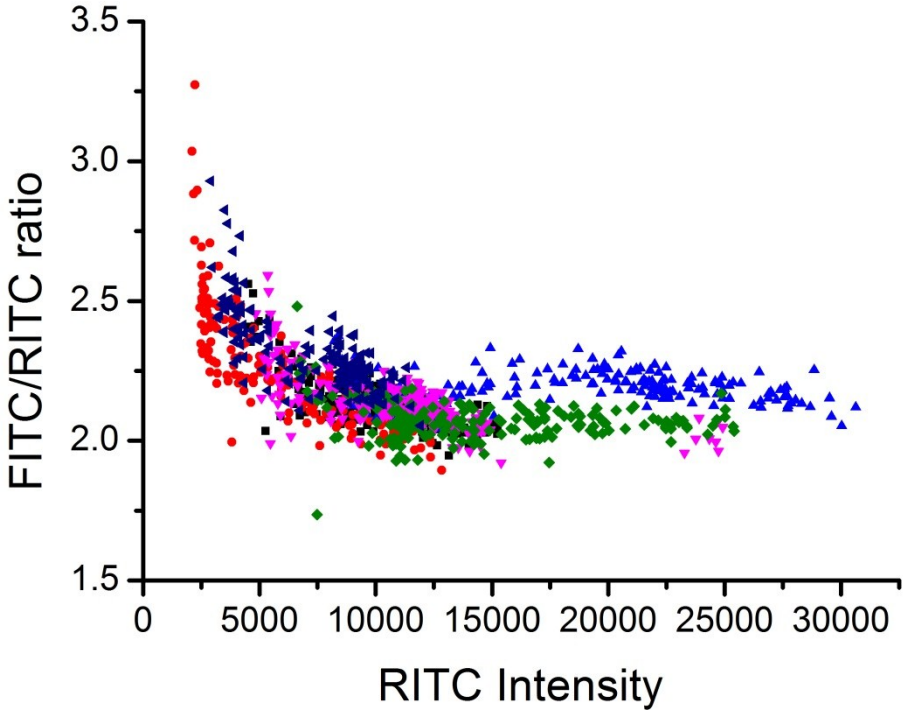


Figure S 9 The relationship between fluorescence intensity of RITC and FITC/RITC ratio. Six FMSNs(+) immobolized on same plane are tracked for a long time to figure out the influence of weak RITC intensity. Based on the results, once the intensity is insufficient (<7000), the FITC/RITC ratio increases, resulting in wrong pH detection. Therefore, the tracking data with weak RITC intensity are discarded before analysis.

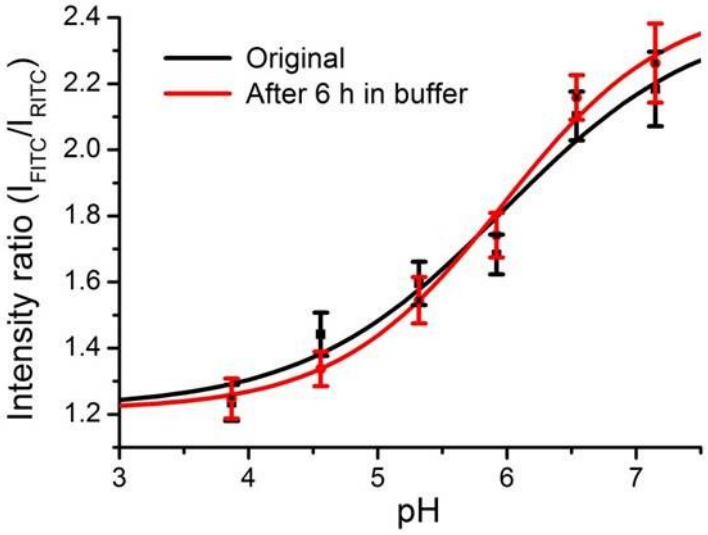


Figure S 10 The pH calibration curves of FMSN(+) before and after being dispersed in buffered solution for 6 h.

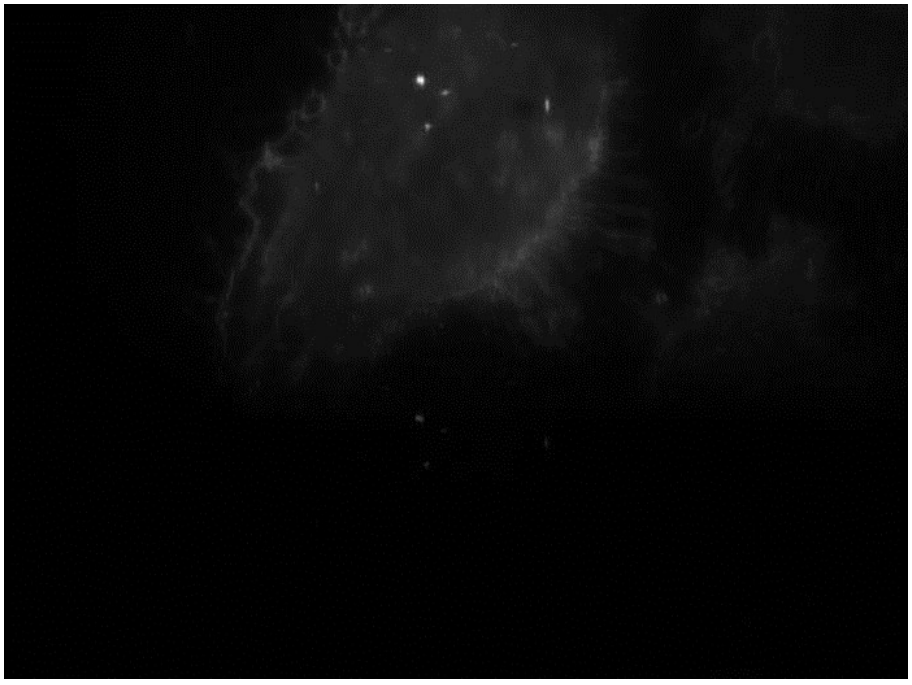


Figure S 11 The location of FMSN(+) in HeLa cell. The membrane of HeLa cell is stained by FM4-64 in order to comfirm that FMSN(+) is located in cell.After single

particle tracking, 10 μL FM4-64 (2 mg in 10 μL HBSS) is added into the sample by pipette, and the signal of FM4-64 is collected by RITC channel.

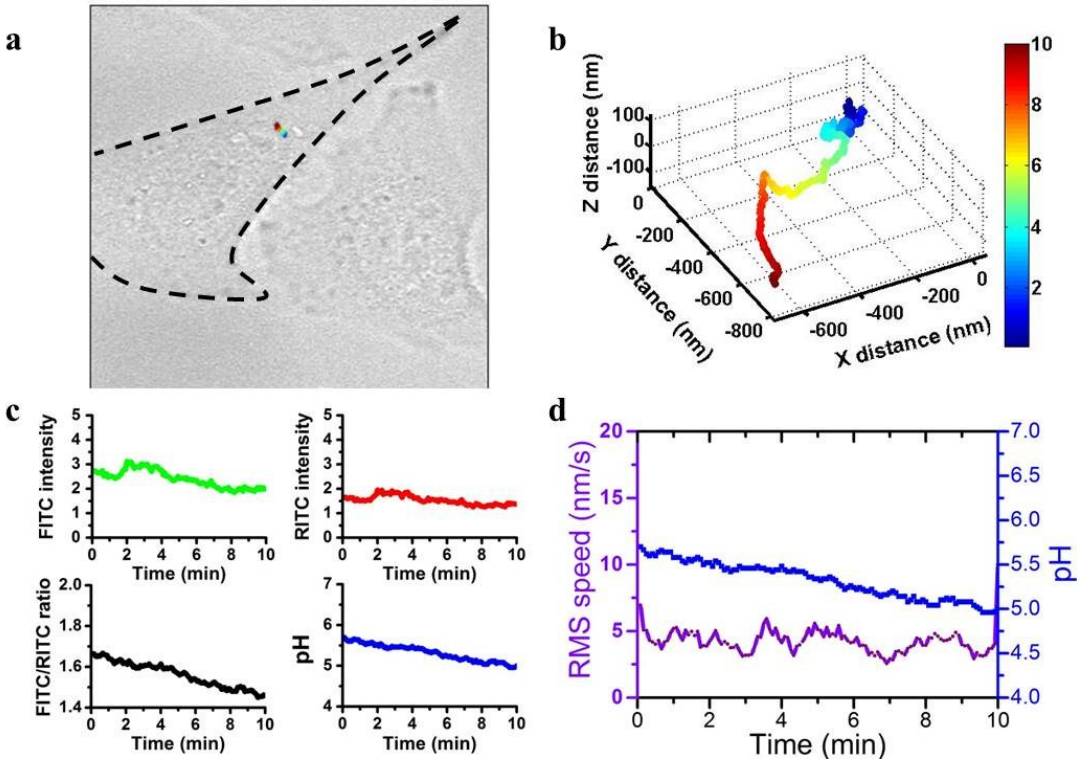


Figure S 12 The trajectory and fluorescent intensity of FMSN(+) as a representative case after 2 h incubation with HeLa cell (one frame per 5 s, tracking for 10 min). (a) The bright field image combined with the trajectory of FMSN(+). (b) The trajectory of FMSN(+).The colorbar represents tracking time (min), indicating that the pathway starts from blue and ends in red. (c) The plots of FITC and RITC intensity, FITC/RITC ratio and local pH. (d) The comparing histogram of local pH and root

mean square velocity.

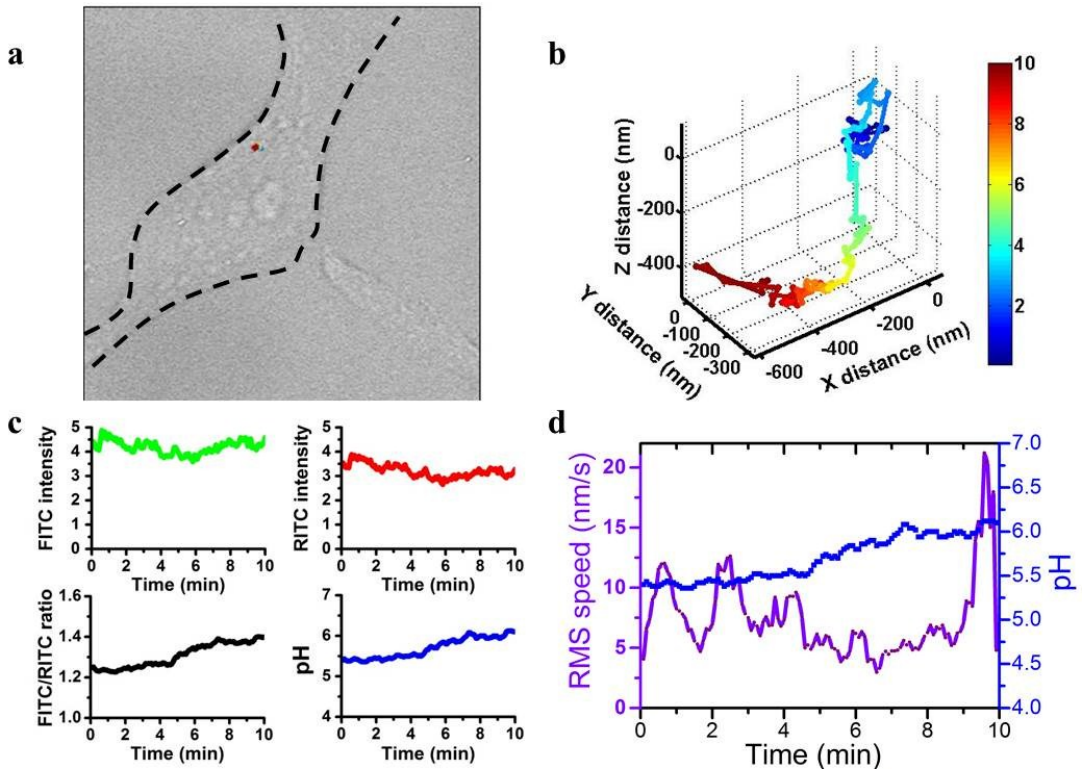


Figure S 13 The trajectory and fluorescent intensity of FMSN(+) as a representative case after 4 h incubation with HeLa cell (one frame per 5 s, tracking for 10 min). (a) The bright field image combined with the trajectory of FMSN(+). (b) The trajectory of FMSN(+). The colorbar represents tracking time (min), indicating that the pathway starts from blue and ends in red. (c) The plots of FITC and RITC intensity, FITC/RITC ratio and local pH. (d) The comparing histogram of local pH and root mean square velocity.

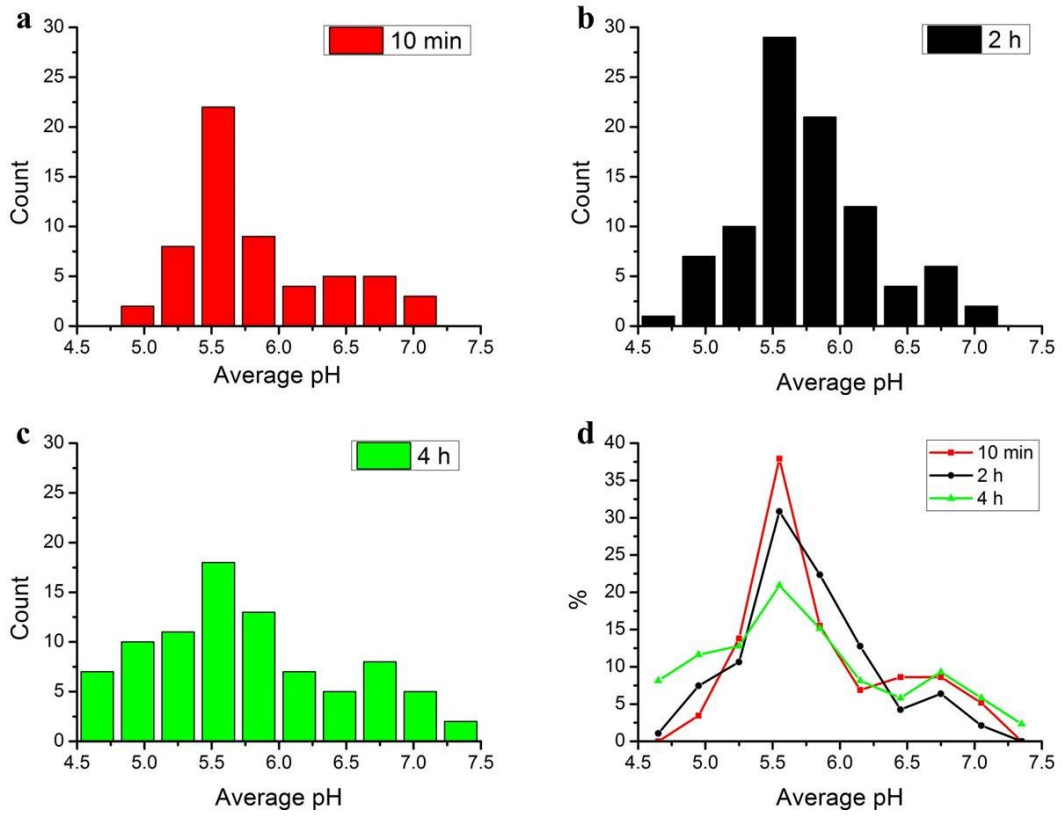


Figure S 14 Histogram of average pH of FMSN(+) classified by incubating time. (a) 10 min. (b) 2 h. (c) 4 h. (d) Comparison of all.

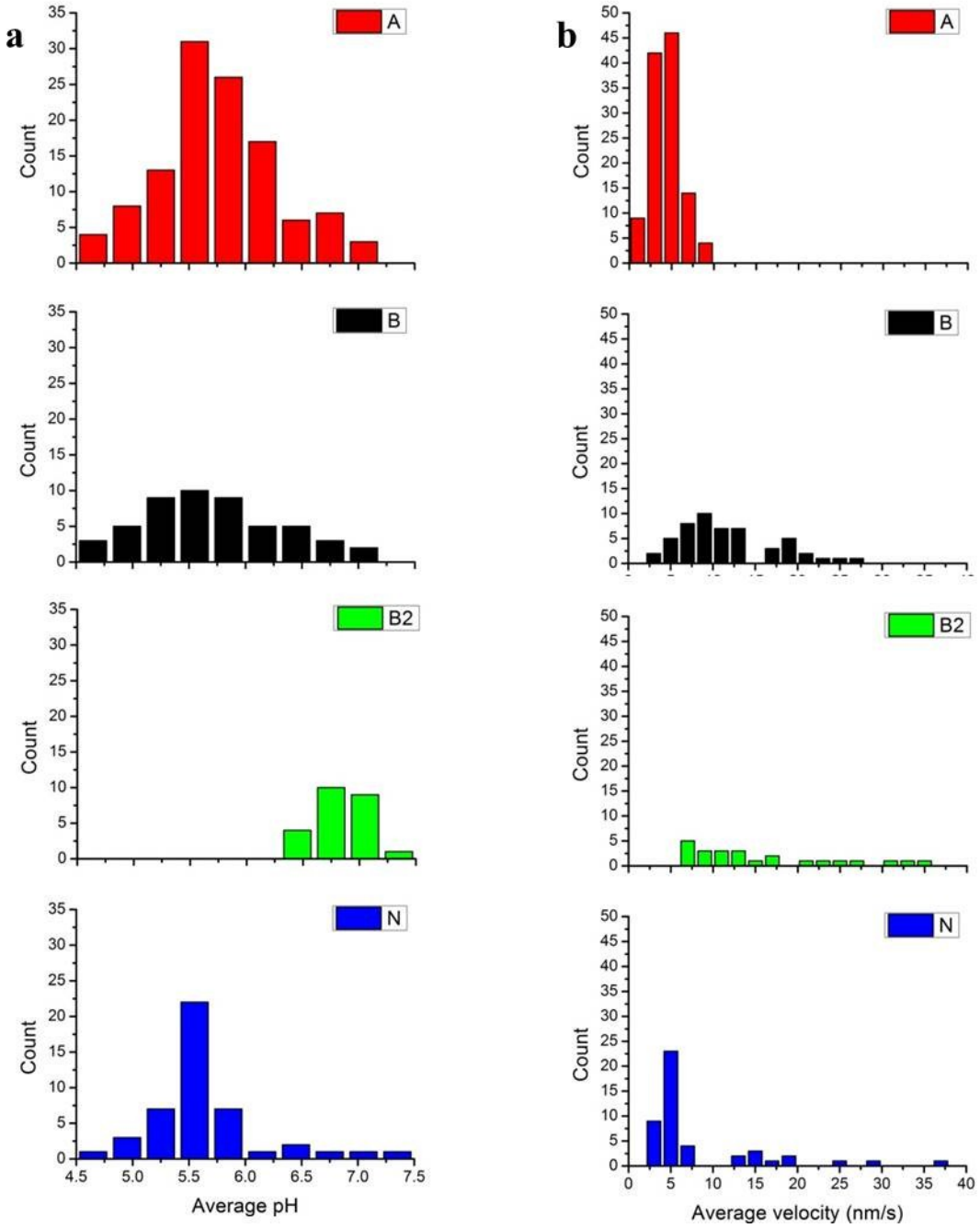


Figure S 15 Histograms of FMSN(+) classified by pH variation types. (a) Average pH. (b) Average velocity.

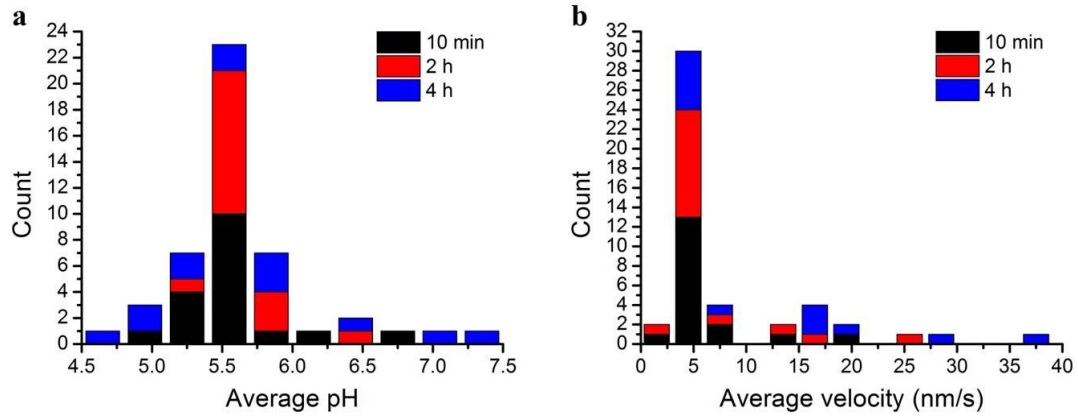


Figure S 16 Histogram of the average local pH (a) and average velocity (b) of type N FMSN(+).

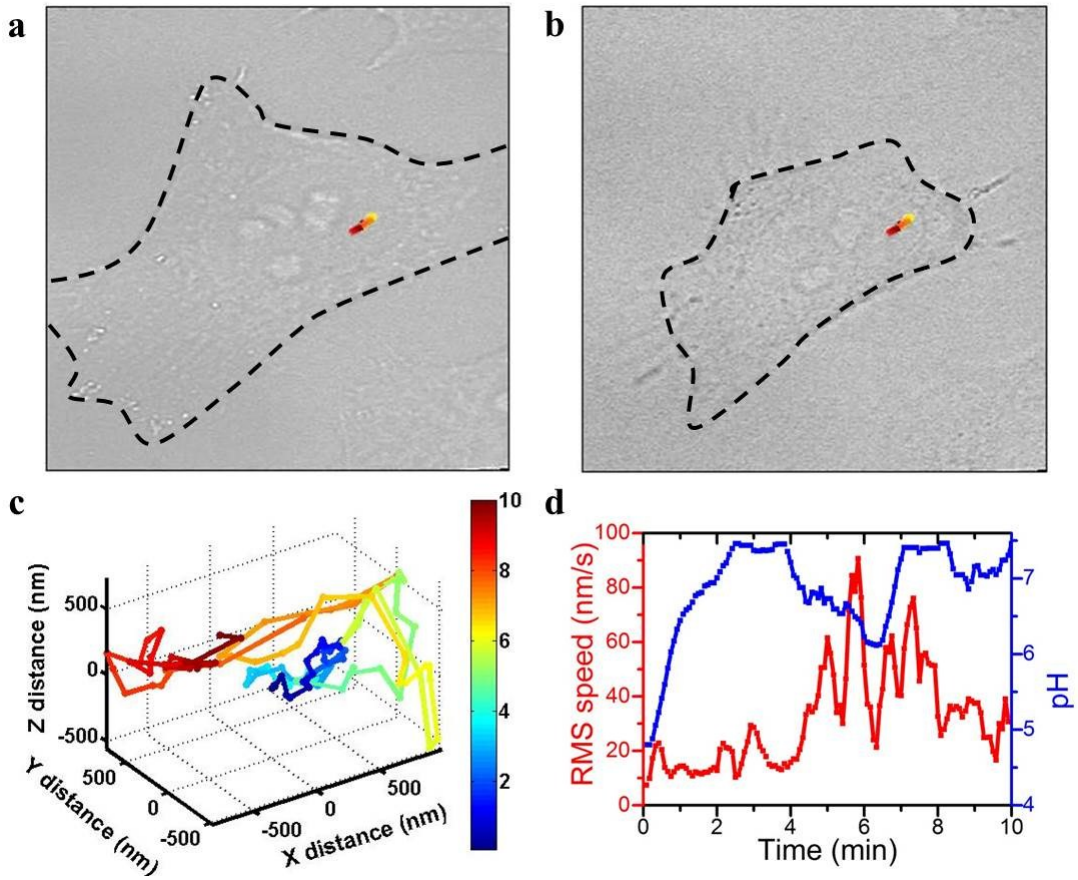


Figure S 17 Tracking the trajectories and local pH of FMSN(+) with the addition of paraquat solution (one frame per 5 s, tracking for 10 min). FMSN(+) is incabated with

HeLa cells for 1 h and the single particle tracking is conducted right after the addition of paraguat solution (details are explained in experimental section). (a) Bright field image of HeLa cell before the addition of paraguat. (b) Bright field image of HeLa cell after single particle tracking experiment. (c) The trajectory of FMSN(+). The colorbar represents tracking time (min), indicating that the pathway starts from blue and ends in red. (d) The comparing histogram of local pH and root mean square velocity.

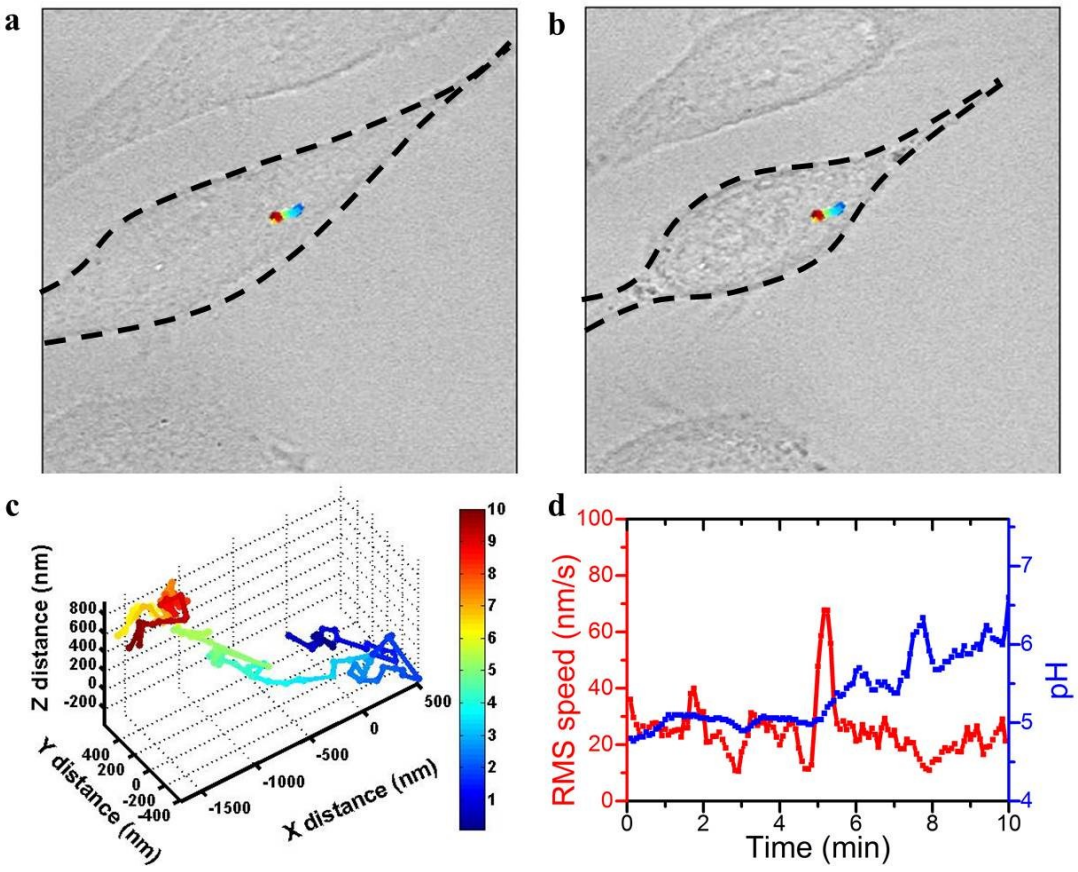


Figure S 18 Tracking the trajectories and local pH of FMSN(+) with the addition of paraquat solution (one frame per 5 s, tracking for 10 min). FMSN(+) is incabated with

HeLa cells for 1 h and the single particle tracking is conducted right after the addition of paraguat solution (details are explained in experimental section). (a) Bright field image of HeLa cell before the addition of paraguat. (b) Bright field image of HeLa cell after single particle tracking experiment. (c) The trajectory of FMSN(+). The colorbar represents tracking time (min), indicating that the pathway starts from blue and ends in red. (d) The comparing histogram of local pH and root mean square velocity.

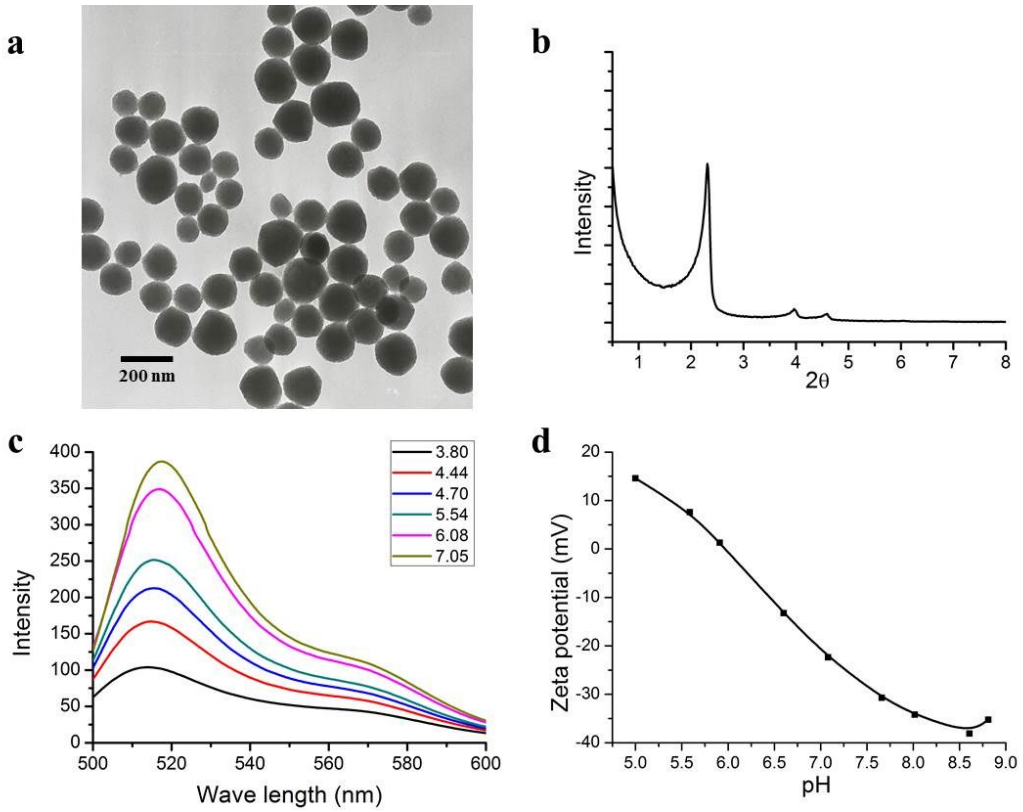


Figure S 19 Material characterization of FMSN(-). (a) TEM image. (b) XRD pattern. (c) Fluorescence spectrum obtained from fluorescence spectrophotometer. The

wavelength of excitation is 473 nm. (d) Zeta potential plot.

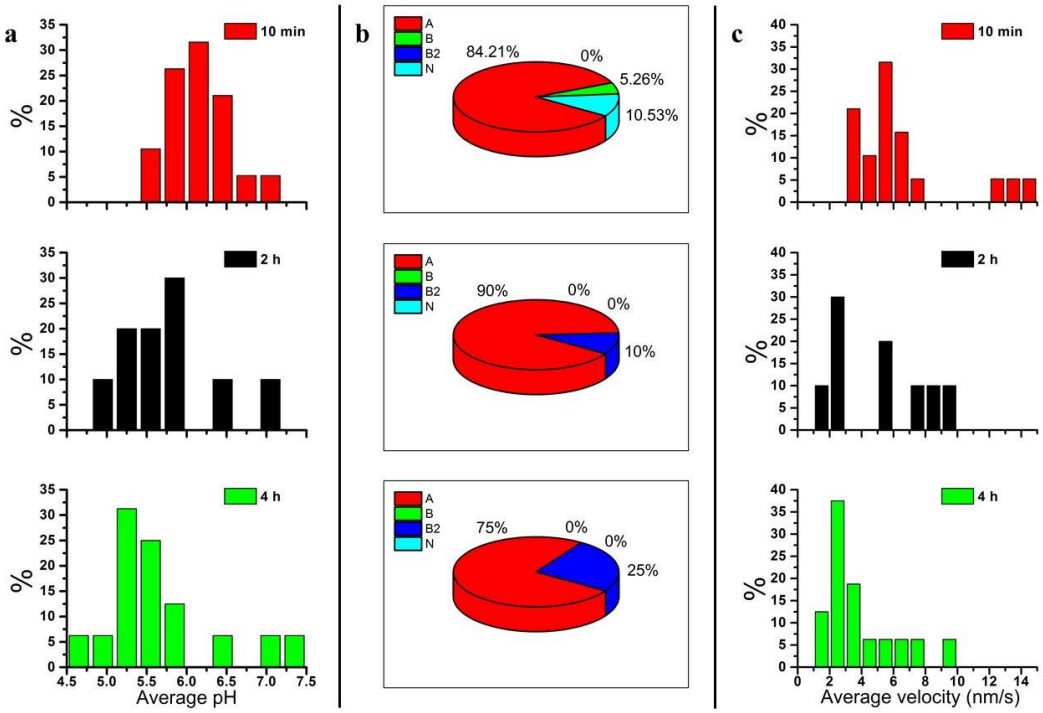


Figure S 20 Histograms of FMSN(-) classified by incubating time. (a) slopes of pH fitting curve (b) pH variation type (c) average velocity. The total numbers of FMSN(-) employed in 10 min, 2 h and 4 h incubation analysis are 20, 11 and 17 respectively.