Electronic Supplementary Material (ESI) for RSC Advances
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Supporting Information

Surface Charge Effect in intracellular localization of Mesoporous Silica Nanoparticles as Probed by Fluorescent Ratiometric pH Imaging

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Supporting information contains experimental procedure and characterization of the materials.

1. Experimental details

Synthesis of FITC&RITC@MSN

Fluorescein isothiocyanate (FITC) 1.5 mg and 3-aminopropyltrimethoxysilane (APTMS) 30 μL were stirred in 5.0 mL EtOH (99%) to form N-1-(3-trimethoxysilylpropyl)-N'-fluoreceylthiourea (FITC-APTMS). tetramethylrhodamineisothiocyanate (RITC) 0.5 mg and 3-aminopropyltrimethoxysilane (APTMS) 10 μL were stirred in 5.0 mL EtOH (99%) to form N-1-(3-trimethoxysilylpropyl)-N'-tetramethylrhodaceylthiourea (RITC-APTMS). Both solutions were prepared in advance and were stirred at room temperature for 24 h. C16TABr (0.58g, $1.64x10^{-3}$mole) and 5 mL of 0.226 M ethanol solution of TEOS (1 mL TEOS in 20 mL 99.5% ethanol) were dissolved in 300 g (0.17 M) aqueous ammonia solution. The stock solution was stirred at 40^{o}C for 5 h. FITC-APTMS, RITC-APTMS and 5 mL of 1.13 M ethanol solution of TEOS (5 mL TEOS in 20 mL 99.5% ethanol) were added with vigorous stirring for 1 h. The solution was then aged statically at 40^{o}C for 24 h. As synthesized samples were then collected by centrifugation at 14000 rpm for 60 min and washed with 95% ethanol twice. FITC&RITC@MSN was stored in 99.5% ethanol.

FITC&RITC Functionalized MSN with Negative Charges

3-Trihydroxysilylpropylmethylphosphonate (PP) 75 μL were stirred in 30 mL EtOH (95%) & 15 mL DI water. PP solution were prepared in advance and were stirred at room temperature for 6 h. 150 mg of FITC&RITC@MSN were re-dispersed in 7 mL of 99.5% ethanol, followed by adding 45mL PP solution. The mixture was heated at 70 ^{o}C for 12 h. Those as-synthesized PP-silanefunctionlized silica nanoparticles were collected by centrifugation and washed with 95% ethanol twice. Surfactant C16TABr was removed by heating in acidic ethanol (1 g 37wt% $HCl_{(aq)}$ in 50 mL 95% ethanol) at 60^{o}C for 24 h followed by centrifugation, and the product was washed with 95% ethanol three times. Final product was stored in 99.5% ethanol.

FITC&RITC Functionalized MSN with Positive Charges

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N-Trimethoxysilylpropyl-N,N,N-trimethylammonium chloride (TA) 140μL were stirred in 30 mL EtOH (95%) & 15 mL DI water. TA solution were prepared in advance and were stirred at room temperature for 6 h. 150 mg of FITC&RITC@MSN were re-dispersed in 7 mL of 99.5% ethanol, followed by adding 45mL TA solution. The mixture was heated at 70 ^{o}C for 12 h. Those as-synthesized TA-silanefunctionlized silica nanoparticles were collected by centrifugation and washed with 95 % ethanol twice. Surfactant C_{16}TABr was removed by heating in acidic ethanol (1 g 37wt% HCl(aq) in 50 mL 95 % ethanol) at 60^{o}C for 24 h followed by centrifugation, and the product was washed with 95% ethanol three times. Final product was stored in 99.5% ethanol.

Powder X-ray Diffraction (XRD)

PANalyticalX'Pert PRO instrument was used for collecting XRD patterns with Cu Kα (λ= 1.5406 Å) radiation in the 2θ range from 0.5^{o} to 8^{o}. The operating current and voltage were at 40 mA and 45 KV.

Nitrogen Adsorption-desorption Isotherms

N_2 adsorption-desorption isotherms were obtained at -196 ^{o}C on Micrometerics ASAP 2010 apparatus. The samples were degassed at 70^{o}C for 24 h under 10^{-3}torr in advance. The specific surface area of samples was calculated based on Brunauer-Emmett-Teller (BET) method and the pore size distribution curves were obtained from the analysis of nitrogen adsorption isotherms using the Barrett-Joyner-Halenda (BJH) method. The pore volume was evaluated at P/Po=0.5 and the textual pore volume was obtained from the difference in pore volume between P/Po=0.99 and P/Po=0.5.

Transmission Electron Microscopy (TEM)

TEM images were taken using a Hitachi H-7100 instrument with an operating voltage of 100 KV. Samples were sonicated to disperse in ethanol and 10 μL of the suspension was dropped to fix on a microgrid.

Dynamic Light Scattering (DLS)

Zeta potentials were obtained at room temperature on MALVEN Nano-ZS. 2 mg of sample suspended in 1mL of H_2O was prepared for measurement. 0.1N NaOH(aq) and 0.1N HCl(aq)were used for titrating the suspension to form a pH gradient from 4.0 to 9.0.

Fluorescence Measurements

Hitachi F-4500 was used for obtaining fluorescence spectra. The excitation wavelength and emission range were set at 490 nm and from 500 nm to 650 nm for FITC, 540 nm and from 550 nm to 650 nm for RITC, respectively. Both excitation and emission slits were 5.0 mm and PMT voltage was 400 V. The pH sensitivity was determined based on the ratio between the sensor peak intensity (fluorescein: 515 nm) and the reference peak intensity (rhodamine: 575 nm) in a series of sodium phosphate buffers from pH 5.0 to 8.5.

Cell Culture

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HeLa cells, a human epithelial cervical cancer cell line, were maintained in Dulbecco's modified eagles medium (DMEM; GIBCO) supplemented with 10% fetal bovine serum (FBS; GIBCO), 100 U/mL penicillin, and 100 μg/mL streptomycin (GIBCO) at 37 ℃ in a humidified and 5% CO_2 atmosphere. When adherent cells reached ~ 60% to 70% confluence, they were detached with 0.25% trypsin-EDTA growth medium to allow for continued passaging.

Flow Cytometry Analysis

Afterincubation at 37°C with various concentrations of MSN-PP and MSN-TA suspended in serum-freemedium for 4 h, the cells were washed in PBS and harvested by trypsinization. After centrifugation, the cell pellets were resuspended in PBS solution. FITC and RITC fluorescence of nanosensors were analyzed by flow cytometry with a FACS Calibur flow cytometry (BD Biosciences).

Confocal Microscope Study

All fluorescence microscopy was conducted using separate excitation lines for FITC (488nm), RITC and FM 4-64 (543nm) of an Argon ion laser. Emission was collected sequentially for FITC (514-535 nm), RITC (550-600 nm) and FM 4-64 (710-800 nm) channels as well as a bright field image.

Endosome Staining of HeLa Cells

FM 4-64 (Molecular Probes) is a classical marker used for staining membrane and endosomes. The cells were co-incubated with MSN-PP or MSN-TA containing 5 μg/mL FM4-64 dye. After 4 h, the supernatants were removed and cells were washed. Fluorescence was observed by confocal microscopy (TCS SP5, Leica).

2. Characterization:

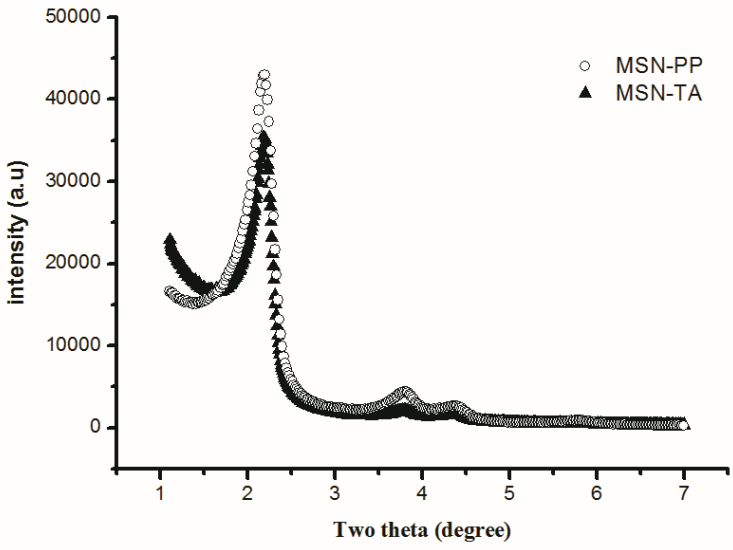

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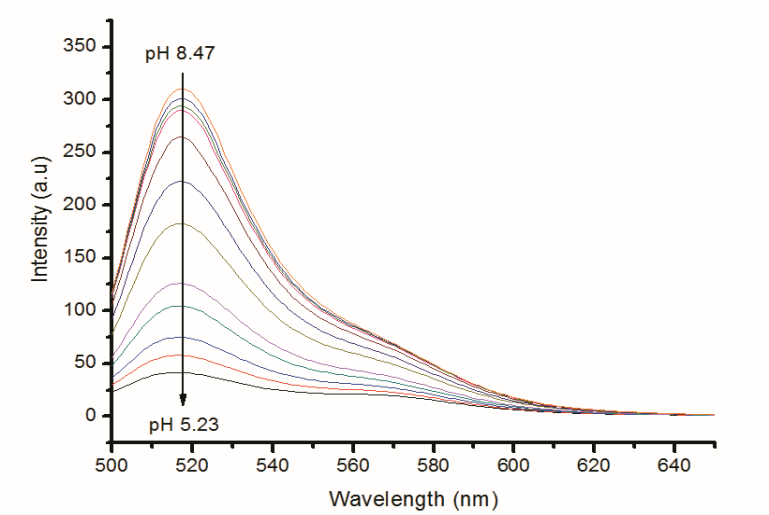
Fig. S1. XRD pattern of MSN-PP and MSN-TA.

a)



b)

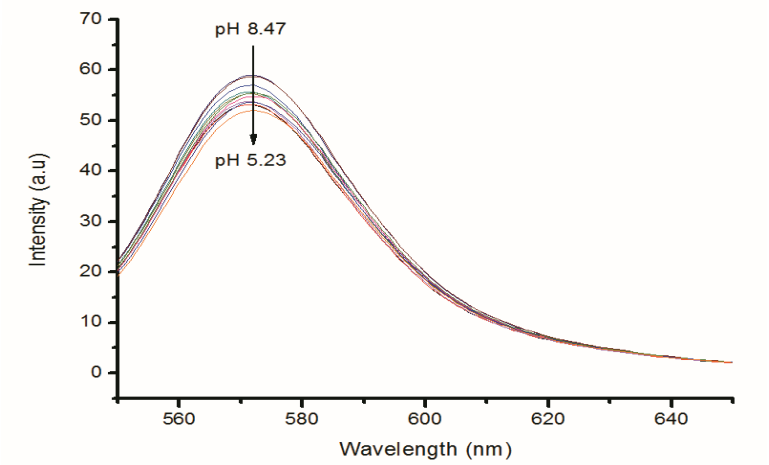


Figure S2. Fluorescence spectra of MSN-PP in sodium phosphate buffers at varying pH values (pH range 5.23 to 8.47). (a) FITC (λ_{ex} = 490 nm), (b) RITC (λ_{ex} = 540 nm).

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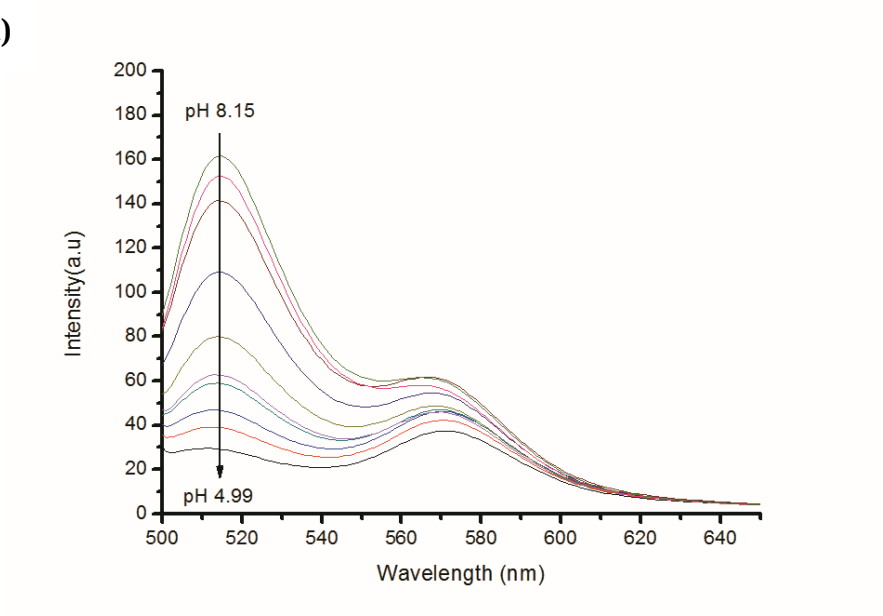
a)



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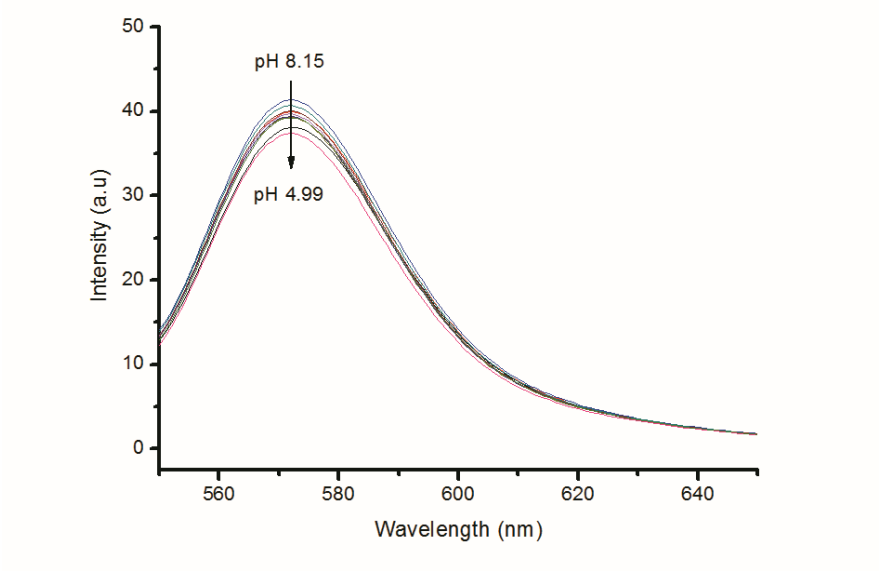

Figure S3. Fluorescence spectra of MSN-TA in sodium phosphate buffers at varying pH values (pH range 5.23 to 8.47). (a) FITC (λ_{ex} = 490 nm). (b) RITC (λ_{ex} = 540 nm).

Notice that in the fuorescence spectra of FITC of MSN-TA, there is a shoulder at 570 nm which is due to FRET effect between the pair of FITC and RITC. In MSN-PP, because the total amount of loaded dye is less, FRET effect is less. But we should note that we never use a 570 nm fluorescence with 490 nm excitation, e.g. the FRET effect is not exploited, unlike ref 28. Thus, the question of FRET is not an issue in our method.