Electronic Supplementary Material (ESI) for Chemical Communications

Dual colored mesoporous silica nanoparticles with pH activable rhodamine-lactam for ratiometric sensing of lysosome acidity

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Supplemenatry materials

Experimental Procedures

LysoTracker Blue DND-22 was purchased from Invitrogen. TNF-α, zVAD, bafilomycin A, and methoxypolyethylene glycol 5,000 acetic acid were purchased from Simga. All other reagents were obtained from Alfa Aesar and used without further purification. Deionized water was used for the preparation of all aqueous solutions. The fluorescence spectra were recorded on a spectrofluorimeter (Shimadzu, RF-5301, Japan). Dynamic light scattering and surface potential analysis of the nanoparticles were performed on Zetasizer Nano ZS (ZEN3500, Malvern).

Cells were analyzed using a fluorescence microscope (Ti-S; Nikon eclipse) equipped with a 100-W mercury lamp (C-SHG1, Nikon). Confocal microscopic images were obtained on LeicaSP2 using the following filters: λex@514 nm and λem@545-580 nm for R6G-lactam; λex@488 nm and λem@510-530 nm for FITC; λex@405 nm and λem@420-480 nm for LysoTracker Blue DND-22. Fluorescence images were merged using Photoshop CS 3.0. Graph by GraphPad Prism5 software. Flow cytometry data were obtained on Becman Coulter. Dual wavelength excitation assays were performed by λex@488 nm for FITC and λex@514 nm for R6G-lactam. The excitation wavelength (488 nm) was used for single-wavength excitation assay. The fluorescence emission of FITC was recorded with filter FL1 (510-535 nm) while that of R6G-amide was recorded by filter FL2 (560-580 nm). 10000 cells were analyzed and the data were analyzed with WinMidi. L929 cells, obtained from American Type Culture Collection, were grown at 37 °C under 5% CO_2 in Dulbecco's Modified Eagle Medium (DMEM, Gibco; Invitrogen) supplemented with 10% fetal bovine serium.

Synthesis of R6G-APTS and FITC-APTS

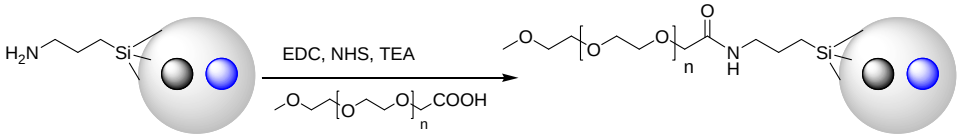
R6G-APTS: Rhodamine 6G (0.2 g) was added in APTS [(3-aminopropyl)triethoxysilane] (10 mL) in a capped vial. The mixture was stirred at room temperature in the dark until the fluorescence of rhodamine 6G disappeared. The resultant product, R6G-APTS, was directly used for the preparation of mesoporous silica nanoparticles. **FITC-APTS**: FITC (0.1g) was added in APTS (5 mL) in a capped vial. The mixture was stirred at room temperature in the dark for 2 hours. The resultant product, FITC-APTS, was directly used for the preparation of mesoporous silica nanoparticles.

Synthesis of R6G-FITC-MSNs

The solutions of R6G-APTS (100 μL) and FITC-APTS (100 μL) were mixed with TEOS (tetraethyl orthosilicate, 2.3 mL) and added to a clean flask containing 240 mL of deionized water, cetyltrimethylammonium bromide (CTAB) (0.5g) and 1.75 mL of sodium hydroxide (2 M) at 80 °C. The mixture was stirred at 80 °C for 2 h and then centrifuged at 10,000 rpm for 30 min to collect the silica MSNs. The MSNs were further treated with mehanol containing HCl (1%) under reflux for 5 hours. The sample was centrifuged and the pellets were further subjected to repeated resuspension in methanol by ultrasonication, centrifugation and decantation to remove the unreacted chemicals and CTAB.

Surface modification of MSNs with polyethylene glycol

The MSNs (200 mg) were aded to anhydrous DMF (20 mL) containing ethyl-dimethylaminopropyl carbodiimide (0.538g), N-hydroxysuccinimide (0.313g), polyethylene glycol (MW 5000, 0.145g) and triethylamine (200 μL). The mixture was sonicated for 90 minutes followed by addition of saturated $NaHCO_3$ solution (30 mL). The mixture was further sonicated for 90 minutes, and then centrifuged. The nanoparticles were collected, extensively washed with deionized water to remove any residual chemcials, and then stored in water (0.1mg/mL) for subsquent characterization and lysosome acidity assays.



Scheme S1. The surfaces of R6G-MSNs were modified with polyethylene glycol polymer to increase the colloidal stability of the nanoparticles.

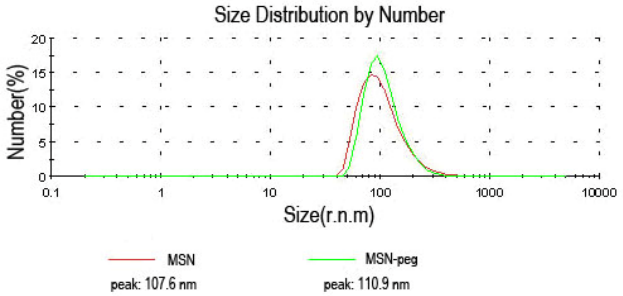


Fig. S1 Determination of diameters of pegylated R6G-FITC-MSNs (in green) and unmodified MSNs (in red) by dynamic light scattering

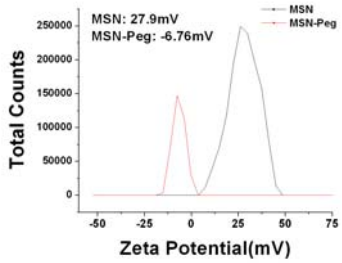


Fig. S2 Zeta potential analysis of surface pegylated R6G-FITC-MSNs as compared to unmodified MSNs

Ratiometric pH titration of R6G-FITC-MSNs under dual wavelength excitation

An aliquot of R6G-FITC-MSNs stock solution (1mg/mL) in water was added to sodium phosphate buffers (200 mM) of various pH values to a final concentration of 1 μg/mL. The fluorescence emission spectra were recorded as a function of pH using λex at 488 for fluorescein and 533 nm for R6G-lactam. The fluorescence emission spectra were recorded as a function of pH.

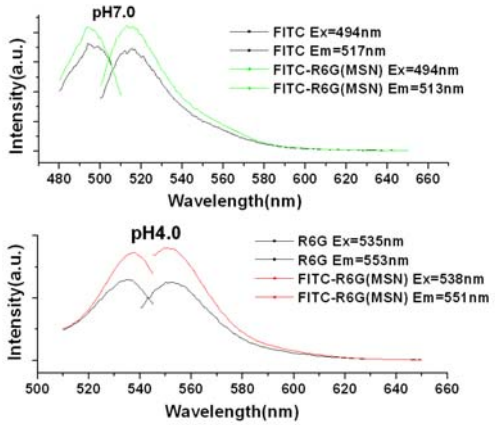


Fig. S3 Comparison of the fluorescence emission spectrum of FITC-R6G-MSNs to fluorescein (pH 7) and rhodamine 6G (pH 4.0).

Ratiometric pH titration of R6G-FITC-MSNs under single wavelength excitation

Aliquots of R6G-FITC-MSNs stock solution in water were added to sodium phosphate buffers of various pH values to a final concentration of 1 μg/mL. The fluorescence emission spectra were recorded as a function of pH using λex at 504 nm. The fluorescence emission spectra were recorded as a function of pH .

Selectivity of R6G-lactam doped in R6G-FITC-MSNs towards representative cations and selected reactive chemical species

To a serial of solutions of R6G-FITC-MSNs (1 μg/ml) in water or DMEM (the cell cultureing medium) was added one of the following cations (1) HClO4 (0.1 mM), NaCl (1 mM), $CaCl_2$ (1 mM), $MgCl_2$ (1 mM), $ZnCl_2$ (1 mM), $CuSO_4$ (1 mM), $CoCl_2$ (1 mM), KCl (1 mM); H_2O_2 (5 mM) or HOCl (5 mM). Fluorescence emission spectra of R6G-lactam on the nanoparticles were recorded using excitation wavelength at 533 nm.

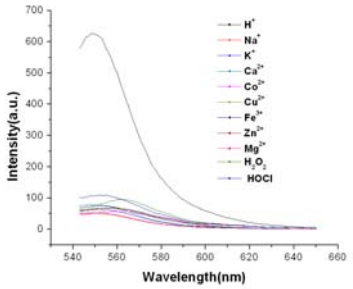


Fig. S4 Fluorescence emission spectra of FITC-R6G-MSNs (0.01mg/mL) to various cations (1mM) and selected reactive chemicals (5 mM) as compared to H^+ (0.1 mM).

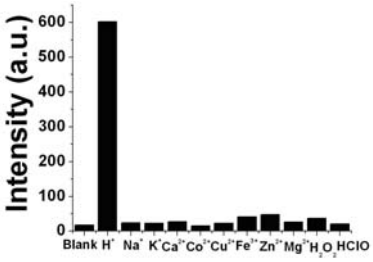


Fig. S5 Selectivity of R6G-FITC-MSN in MEM (cell culture medium) for H^+ (0.1 mM) over H_2O_2 (5mM), HOCl (5mM) or various cations (1 mM) (λex:533 nm, λem:553nm).

Endocytosis of R6G-FITC-MSNs into lysosome in live cells

L929 cells were seeded on 35 mm glass-bottom dishes (NEST) and incubated for 24 hours, followed by addition of LysoTracker Blue DND 22 (1 μM) and dye doped silica nanoparticles (10 μg/mL). The cells were further incubated for 30 minutes. The medium was removed and replaced with fresh medium. For reversal staining of lysosomes, cells were pre-incubated for 4 h at 37 °C in the absence or presence of 50 nM bafilomycin A1, and then incubated in media supplemented with nanoparticles (10 μg/mL) or LysoTracker Blue DND 22 (1 μM) for 30 min. Cells were then analyzed with confocal fluorescence microscope or flow cytometry.

pH titration curve in vivo.

L929 cells were cultured with medium containing FITC-R6G-MSNs (0.05mg/mL) for 2 hours, and then cells were digested from culture dish and washed with PBS for 2 times. Cells were then incubated in Briton Buffer of different pH containing nigericin (10 μM) for 10 minutes. Cells were then analyzed with flow cytometry.

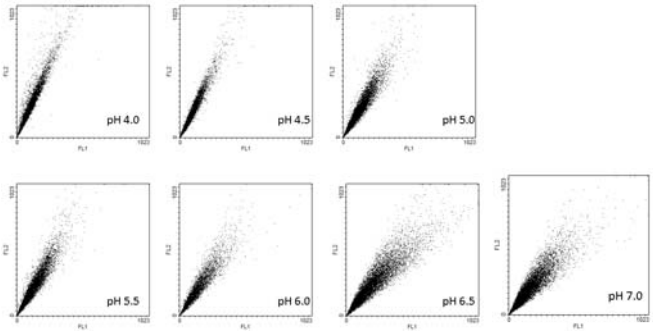


Fig. S6 Calibration curve of ratiometric pH response of R6G-FITC-MSNs in lysosomes of L929 cells by flow cytometry.

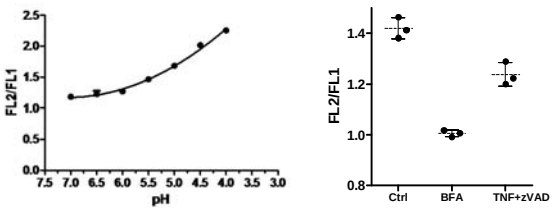


Fig. S7 Calibration curve of ratiometric pH response of R6G-FITC-MSNs in lysosomes of L929 cells by flow cytometry.

Ratiometric pH assay of lysosome acidity in L929 cells

L929 cells were cultured with medium containing FITC-R6G-MSNs (0.05mg/mL) for 2 hours. Then medium was removed. The cells were washed with PBS for 2 times and then replaced in fresh medium (DMEM with 10% FBS from Gibco) for half an hour. Cells were then treated with medium containing 50nM bafilomycin A for 4 hours. After that, cells were analysed with flow cytometry.

Effects of incubation time of cells with R6G-FITC-MSNs on lysosomeal pH

To check the effects of incubation time on the entry of R6G-FITC-MSNs into cells. L929 cells were cultured with nanoparticles ((0.05mg/mL). At fixed ponit time, the cells were analyzed with flow cytometry. It was shown that the amount of nanoparticles internalized in to cells increased as a funtion of time, while the lysosomal pH (FL2/FL1 ratio) remain unaffected.

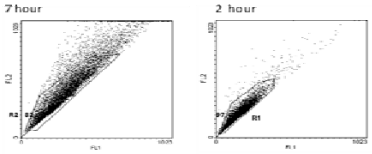


Fig. S8 Effects of incubation time on the accuracy of R6G-FITC-MSNs based assay of lysosome acidity in L929 cells.

Assay of lysosome pH of L929 cells undergoing apoptosis with R6G-FITC-MSNs.

L929 cells were treated with TNF-α (10 ng/mL) and z-VAD (20 nM) for 6 hours, and then cultured with FITC-R6G-MSNs (0.05mg/mL) for 2 hours. The healthy cells and the apoptotic cells were separately gated and analyzed with flow cytometry.

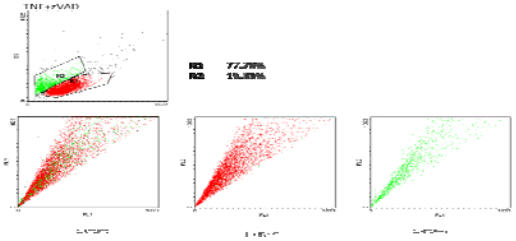


Fig. S9 Comparison of the lysosome pH of Apoptotic L929 cells (in green) as comapred to healthy cells via ratiometric fluorescence emission of R6G-FITC-MSNs.