Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B

Supporting Information

A Broad-range fluorescent pH Sensor Based on Hollow Mesoporous Silica Nanoparticles with Surface Curvature Effect

*Chieh-Jui Tsou, Chia-yin Chu, Yann Hung and Chung-Yuan Mou**

Synthesis of Dye-MSN

Initially, 0.29 g of CTAB was dissolved in 150 g of 0.51 M ammonium hydroxide and then 0.53 mmole TEOS in 2.5 mL ethanol was added. The solution was sealed and heated to 40 oC with continuous stirring. After four hours, 2.5 mL APTMS-conjugated FITC/ethanol solution was added to the mixture, followed by the addition of 2.21 mmole TEOS in 2.5 mL ethanol solution and stirred for one hour. 0.625 mL APTMS-conjugated RITC/ethanol solution was added and the stirring was continued for one hours then stopped. After the solution was aged 24 hours at 40 oC, as-synthesized precipitates were centrifuged to isolate the mesoporous silica materials. The products were washed by ethanol four times with sonication to remove unreacted chemicals. In order to remove the surfactant templates and avoid dye leakage, the precipitates were dispersed in ammonium nitrate/ ethanol solution (6 g/ 50 mL) and heated at 60 oC for one hour. After centrifugation to collect the particles, the dye-MSN was washed by ethanol three times, then dispersed in ammonium nitrate/ ethanol (3 g/ 50 mL) solution and heated at 60 oC for another one hour. After centrifugation and washed by ethanol for three times, the nanoparticles were dispersed in 99.5% ethanol for storage.

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 Dye-HMS

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 dye-HMS 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).

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. All images were acquired with a 40x oil DIC objective. Differential interference contrast (DIC) images were collected simultaneously using a 488 nm argon-ion laser.

Establishment of Calibration Curves

For the calibration curve established by fluorescence spectrum, the dye-loaded nanoparticles were dispersed in a series of sodium phosphate buffer (ion strength = 0.05 M) from pH 4 to 9.5. The calibration curve is obtained by collecting the emission intensity of FITC at 514 nm (λ_{exc} = 473 nm) and RITC at 576

nm (λ_{exc} = 544 nm). The ratio of emission intensity (I_F/I_R) was plotted with pH. For the confocal microscope-established one, the dye-HMS was dispersed in sodium phosphate buffer (ion strength = 0.05 M) with desired pH. The ratio of intensity (I_F/I_R) was derived from the sum of fluorescence intensity of the regions of interest (ROIs) in FITC and RITC channels. The background subtraction was conducted by first choosing a ROI without any pH sensor and evaluating the average fluorescence intensity of pixels for every confocal image which was defined as the background level. For each confocal image, the background level was then subtracted from every pixel of ROIs containing pH sensors. The ratio of intensity (I_F/I_R) was calculated from over thirty different ROIs for each pH.

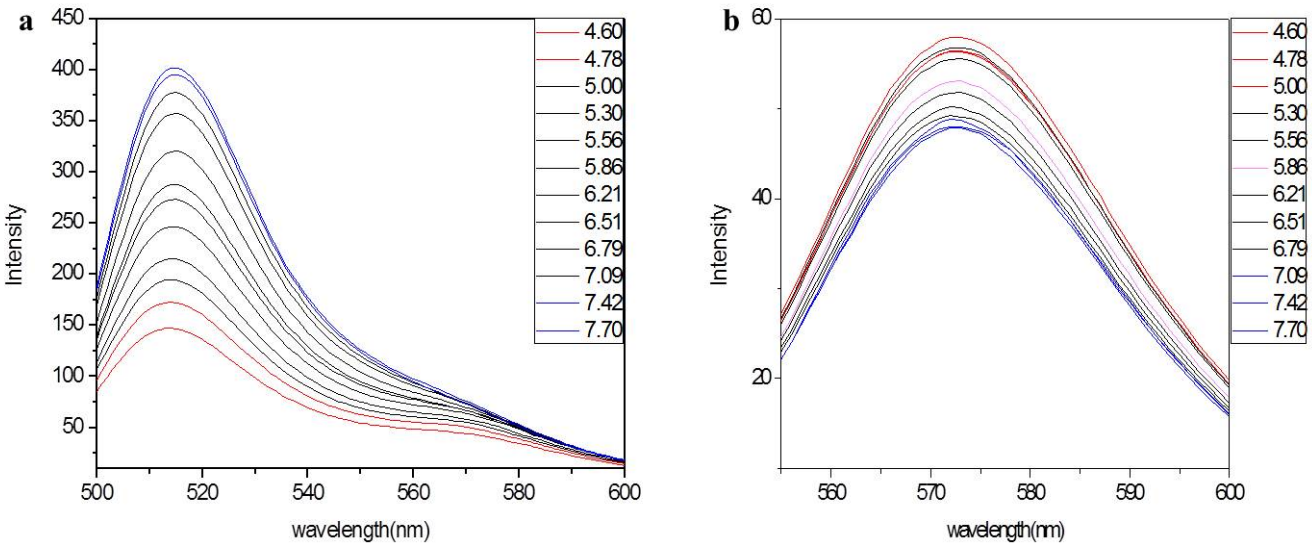

Figure S1. Fluorescence spectra of dye-MSN. (a) FITC (b) RITC

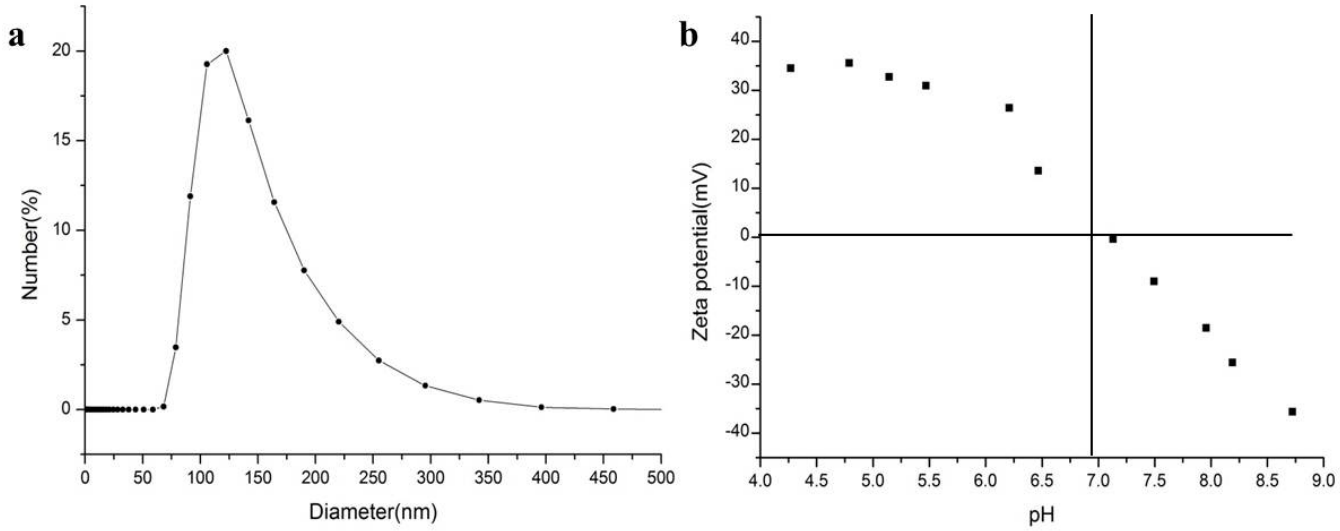

Figure S2. (a) The size distribution of dye-HMS by dynamic light scattering (DLS). (b) Zeta potential versus pH diagram of dye-HMS.