

Electronic Supplementary Information (ESI)

Allyl iodide, 7-hydroxy-coumarin, fluorescein, rhodamine B, cesium carbonate, trimethoxysilane (TMS), triethoxysilane (TES), platinum(0)-1,3-divinyl-1,1,3,3-tetramethyldisiloxane complex solution, Pt(dvs), and platinum on activated charcoal, Pt/C, were purchased from Aldrich, and tetraethyl orthosilicate (TEOS) was purchased from TCI. N-trimethoxysilylpropyl-N,N,N-trimethylammonium chloride, (MeO)₃Si-PTMA, and Methyl(polyethyleneoxy)-propyl-trimethoxy-silane, (MeO)₃Si-PEG, were purchased from Gelest. They were used without further purifications. N,N-dimethylformamide, DMF, methanol and toluene were dried for the removal of water. Silica gel for column chromatography was purchased from Merck. All reactions were carried out under nitrogen. The synthetic dye was characterized by ¹H- and ¹³C-NMR (Bruker, 300 MHz and Varian, 500 MHz) and Mass spectrometer (JEOL, JMS-AX505WA). The size and shape of silica nanoparticles were characterized by transmission electron microscope, TEM (Hitachi, H-7600) and field-emission scanning electron microscope, FE-SEM (Hitachi, S-4300). The absorption and emission of fluorescent silica nanoparticles were measured by UV-Visible spectrometer (Sinco, S-3100) and Acton spectra pro with a 500W Xenon light source.

Synthesis of allyloxy-rhodamine B, A1

0.5 g (1.04 mmol) of rhodamine B, 0.54 g (3.12 mmol) of allyl iodide, and 1.02 g (3.12 mmol) of cesium carbonate were mixed in 30 mL of DMF, and the mixture was stirred at 60°C for a day. The mixture was extracted with methylene chloride and water 3 times. The methylene chloride layer was collected and concentrated by rotary evaporator, and then it was dried in vacuum. A dark reddish powder product (0.511 g, 94.6 % yield) was isolated by a column chromatography (SiO₂, eluent; methylene chloride/ methanol = 10/1). ¹H NMR (300 MHz, CDCl₃); δ ppm 8.31 (d, 1H), 7.82 (tt, 2H), 7.34 (d, 1H), 7.10 (d, 2H), 6.93 (dd, 2H), 6.80 (d, 2H), 5.70 (m, 1H), 5.20 (dd, 2H), 4.53 (d, 2H), 3.68 (q, 8H), 1.34 (t, 12H). ¹³C NMR (300 MHz, CDCl₃); δ ppm 164.61, 158.62, 157.65, 155.45, 133.48, 133.20, 131.28, 131.19, 131.04, 130.41, 130.18, 129.81, 119.04, 114.30, 113.43, 96.21, 66.02, 46.23, 12.70. HR-MS(FAB+) Calcd. for 483.2642 m/z, Obsd. for 483.2640 m/z

Synthesis of diallyloxy-fluorescein, B1

0.5 g (1.5 mmol) of fluorescein, 0.77 g (4.5 mmol) of allyl iodide, and 1.47 g (4.5 mmol) of cesium carbonate were used. The reaction conditions and purification steps were similar to A1. 0.560 g (90.2 % yield) of orange powder product was obtained by a column chromatography (SiO₂, eluent; methylene chloride/ methanol = 20/1). ¹H NMR (500 MHz, CDCl₃); δ ppm 8.27 (d, 1H), 7.70 (tt, 4H), 7.32 (d, 1H), 6.97 (d, 1H), 6.91 (q, 2H), 6.77 (dd, 1H), 6.55 (d, 1H), 6.45 (s, 1H), 6.05 (m, 1H), 5.60 (m, 1H), 5.40 (dd, 2H), 5.11 (dd, 2H), 4.66 (d, 2H), 4.47 (m, 2H). ¹³C NMR (500 MHz, CDCl₃); δ ppm 185.95, 165.26, 163.22, 159.16, 154.44, 150.22, 134.65, 132.93, 132.14, 131.47, 131.19, 130.80, 130.75, 130.46, 130.21, 129.93, 129.90, 129.15, 119.43, 118.99, 117.99, 115.22, 114.06, 114.02, 106.01, 101.44, 101.40, 69.69, 66.28 HR-MS(EI+) Calcd. for 412.1311 m/z, Obsd. for 412.1312 m/z

Synthesis of 7-allyloxy-coumarin, C1

1 g (6.17 mmol) of 7-hydroxy-coumarin, 3.12 g (18.6 mmol) of allyl iodide, and 6.06 g (18.6 mmol) of cesium carbonate were mixed in 30 mL of DMF, and the mixture was stirred at 60°C. After 1 day, the mixture was extracted with methylene chloride and water 3 times. The methylene chloride layer was collected and concentrated by rotary evaporator, and then it was dried in vacuum. The crude product was dissolved in the smallest amount of methanol and precipitated into the large amount of water. The product was obtained by filtration and washed with water. After drying in vacuum, 1.13 g (90.6 % yield) of yellowish-white powder product was obtained. ¹H NMR (500 MHz, CDCl₃); δ ppm 7.65(d, 1H), 7.38(d, 1H), 6.86(dd, 1H), 6.81(d, 1H), 6.25(d, 1H), 6.05(m, 1H), 5.45(d, 1H), 5.33(d, 1H), 4.60(dd, 2H). ¹³C NMR (500 MHz, CDCl₃); δ ppm 161.97, 161.39,

156.01, 132.37, 129.30, 118.74, 113.35, 113.28, 112.86, 101.94, 69.48
70 HR-MS(FAB+) Calcd. for 202.0630 m/z, Obsd. for 203.0705(M+1) m/z

Hydrosilation of the allylated dyes, A2, B2, C2

All the hydrosilation was carried out in a similar manner.² As a representative example, 50 mg (0.096 mmol) of A1, 25 mg (0.192 mmol) of TMS, and catalytic amounts of Pt/C was mixed in 20 mL of a freshly dried methanol. After refluxing for 1 day, the catalyst was removed by a filtration over Celite. For a synthesis of C2, the reported method from Shea's group was slightly modified.³

General method of synthesizing 50 nm-sized fluorescent silica nanoparticles

30 mg of the modified fluorescent dyes (A2, B2, or C2) and 0.86 g of tetraethyl orthosilicate (TEOS) were dissolved in a dried ethanol and 1.0 ml of ammonia and 1.0 ml of water were added with stirring. After 12 hours of stirring, fluorescent silica nanoparticles were isolated by centrifugation at the speed of 15,000 rpm, and the supernatant was removed. The isolated products were redispersed in ethanol. The washing and re-dispersion was repeated 3 times. Finally, the nanoparticle solution was centrifuged at the speed of 4,000 rpm to remove any aggregated particles. The purified fluorescent silica nanoparticles were homogeneously dispersed in ethanol, water, or buffer. The size and shape of the nanoparticles were characterized by TEM and FE-SEM. Synthetic procedures were similar for different size fluorescent silica nanoparticles, except the concentration of TEOS, water, and ammonia. Table S1 summarizes the conditions for silica nanoparticles of various sizes.

Surface modification of fluorescent silica nanoparticles

30 mg of the modified fluorescent dyes (A2, B2, or C2) and 0.86 g of tetraethyl orthosilicate (TEOS) were dissolved in a dried ethanol and 1.0 ml of ammonia and 1.0 ml of water were added with stirring. After 12 hours of reaction, without isolating fluorescent silica nanoparticles, surface modification was carried out by direct adding of (MeO)₃Si-PTMA or (MeO)₃Si-PEG (4.32 g; about 20 times excess amount) and stirring for another 20 hours. Surface-modified fluorescent silica nanoparticles were collected by a centrifugation, and the supernatant was removed. The isolated products were redispersed in water, and this washing and re-dispersion were repeated 3 times.

Quantum yield⁴

The relative quantum yields (QY_{rel}) were measured based on the known method.^{4a} 7-Hydroxycoumarin, fluorescein, rhodamine B, SiO₂(Cou)-OH, SiO₂(Flu)-OH, and SiO₂(RhB)-OH were dissolved in EtOH, and absorption and emission spectra were taken. A graph of integrated PL intensity vs. absorbance was plotted, and the result should be a straight line with gradient m, and 0 intercept value. After getting the gradients of the graphs, relative values are calculated using the standard samples which have a fixed and known PL quantum yield value.^{4b-d}

	SiO ₂ (Cou)-OH	SiO ₂ (Flu)-OH	SiO ₂ (RhB)-OH
QY _{rel}	0.025	0.311	0.184

Photostability test⁵

Fluorescein, rhodamine B, SiO₂(Cou)-OH, SiO₂(Flu)-OH, and SiO₂(RhB)-OH were dissolved in 3 mL of ethanol, having almost same UV absorbance by varying the amount. Samples were exposed to UV light under a W-Halogen lamp (200W, KANDOLITE[®]) with light intensity of 757 mW/cm². Emission spectra for all the samples were taken after appropriate illumination times.

Subculture of MC3T3-E1 cells

The murine calvaria-derived osteoblast MC3T3-E1 cells have been described previously.⁶ Cells were grown in αMEM and supplemented with 10% fetal bovine serum (FBS), 50 U/ml penicillin, 50 μg/ml streptomycin and 2 mM L-glutamine (Growth medium). Cell culture medium, αMEM was purchased from Irvine Scientific (Santa Ana, CA), FBS from Atlanta Biologicals (Atlanta, GA) and penicillin, streptomycin and L-Glutamine from Invitrogen Corp. (Carlsbad, CA). To determine the

effect of subculturing, cells containing nanoparticles were treated with 1 ml of trypsin (Mediatech Inc., Herndon, VA). The trypsinized cells in the medium were divided into half with 2 plates. Each plate was incubated until cells covered the plate. Every trypsinized plate was monitored by fluorescent microscope.

Measurement of cell viability

All cells were cultured at 37°C with 5% of CO₂. MC3T3-E1 (murine preosteoblast), A549 (human lung cancer), and HEK 293 (human embryonic kidney) cells were grown in the following medium; MC3T3-E1 with cell alpha-MEM and 10% FBS, A549 with DMEM (Mediatech Inc., Herndon, VA) and 4% FBS, and HEK 293 with DMEM and 10% FBS, respectively. All mediums contained antibiotics and glutamine. The cell viability was measured for all cell types using a well-known MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfonyl)-2H-tetrazolium, inner salt) assay according to manufacturer's protocol (Promega, Madison, WI). Cells were plated at 5x10³ cells/100µl per well in 96 well plate. Twenty-four hours after plating nanoparticles were added as indicated. The change in absorbance was measured 1 hour after addition of XTT assay reagent on a BioRad Lumimark plate reader (BioRad Laboratories, Hercules CA).

References

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Entry ^(a)	Molar concentration (mol/L)			Average size (nm) ^(c)	Reaction time (h)
	TEOS	Ammonia	Water ^(b)		
1	0.054	0.180	2.000	30	12
2	0.072	0.180	2.000	50	12
3	0.152	0.180	3.778	100	4
4	0.152	0.355	4.000	200	4
5	0.152	0.533	4.222	300	4
6	0.152	0.533	7.778	500	4

Table S1 Reaction conditions of size-controlled silica nanoparticles; (a) stirring rate of 1 and 2 was at 400 rpm, and the others were at 250 rpm. (b) The concentration included water in 28% of ammonia. (c) Their size was characterized by TEM or FE-SEM. (See Figure S1)

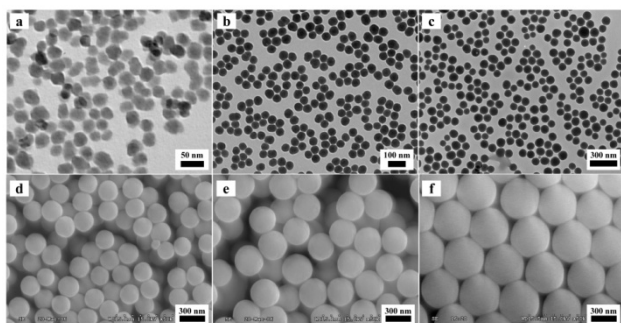


Figure S1 TEM (a, b, c) and FE-SEM (d, e, f) images of silica nanoparticles; (a) 30 nm, (b) 50 nm, (c) 100 nm, (d) 200 nm, (e) 300 nm, and (f) 500 nm

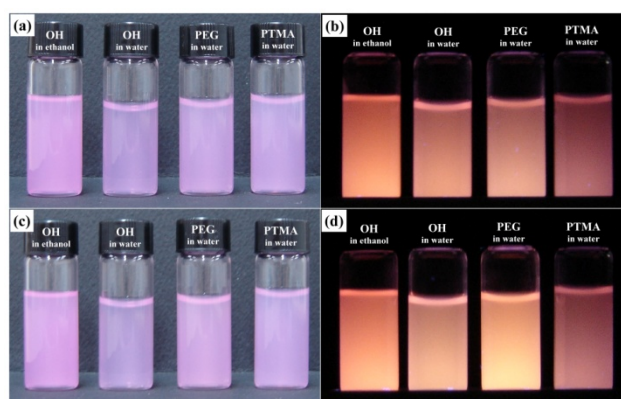


Figure S2 Photographs of silica nanoparticles having various surfaces before (a and b) and after (c and d) reflux in ethanol and water; Images of (b) and (d) were taken under UV irradiation.

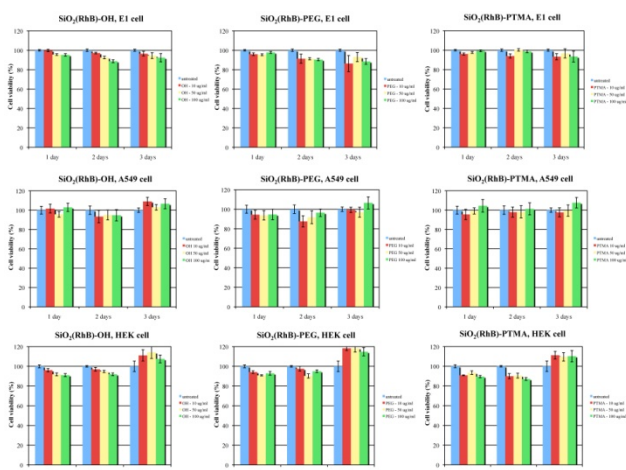


Figure S3 Cell viability in three kinds of cell lines by MTS; 50 nm-sized, red-fluorescent silica nanoparticles were treated, depending on periods, dose, and surface charge. The blue is untreated, the red is 10 µg/ml of silica nanoparticles-treated, the yellow is 50 µg/ml of silica nanoparticle-treated, and the green is 100 µg/ml of silica nanoparticles-treated.

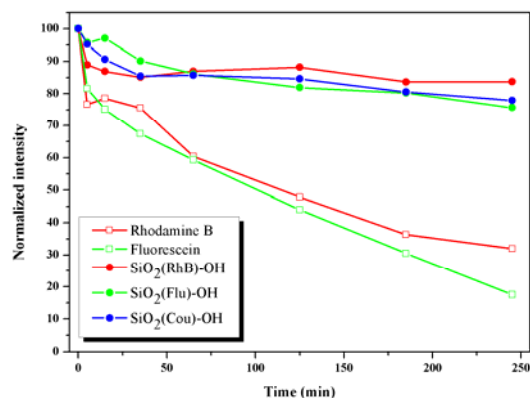


Figure S4 Photo-bleaching experiment; Fluorescence emission spectra were taken after 0, 5, 15, 35, 65, 125, 185, and 245 min of UV exposure. After 245 min of continuous illumination, the PL intensities of silica nanoparticles were remained about 80% of original values for SiO₂(Cou)-OH, SiO₂(Flu)-OH, and SiO₂(RhB)-OH, comparing to 18% and 32% for fluorescein and rhodamine B, respectively.