

Supporting Information

**Light-Induced and Redox-Triggered Uptake and Release of Substrates to and
from Mesoporous SiO₂ Nanoparticles**

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Experimental

Materials

Tetraethyl orthosilicate (TEOS), (3-aminopropyl) triethoxysilane (APTES) and 2,3-dichloro-1,4-naphthoquinone were purchased from Aldrich. Hexadecyltrimethylammonium bromide (CTAB), dimethyl sulfoxide (DMSO), N-hydroxysuccinimide (NHS), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide (EDC), rhodamine B, eosin Y, ascorbic acid (AA), methyl viologen (MV^{2+}), β -nicotinamide adenine dinucleotide reduced dipotassium salt (NADH), doxorubicin hydrochloride and 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid sodium salt (HEPES) were purchased from Sigma. All other chemicals were of analytical grade, and ultrapure water from a NANOpure Diamond (Barnstead Int., Dubuque, IA) source was used throughout the experiments.

Instrumentation

Fluorescence measurements were performed using a Cary Eclipse device (Varian Inc.). The excitation wavelengths of rhodamine B, eosin Y and doxorubicin dyes were 561 nm, 533 nm and 494 nm, respectively. UV-vis absorption spectra were recorded using a Shimadzu UV-2401 spectrophotometer. Surface areas were determined using a Nova 1200e BET meter (Quantachrome Instruments, USA) by nitrogen adsorption/desorption at the temperature of liquid nitrogen. SEM images were taken by a Magellan 400L scanning electron microscope.

Synthesis of mesoporous silica nanoparticles

Amino-functionalized mesoporous SiO₂ NPs were prepared according to a previously reported procedure.¹ The SiO₂ NPs were precipitated, washed with distilled water and methanol, and dried in air. To remove N-cetyltrimethylammonium bromide, (CTAB), the NPs were refluxed for 16 hours in a solution composed of HCl (37%, 1.00 ml) and methanol (80.00 ml), and were, then, extensively washed with distilled water and methanol. The resulting, CTAB-free, amino-functionalized mesoporous SiO₂ NPs were placed in vacuum to remove the remaining solvent from the pores.

SiO₂ NPs without pores were synthesized according to a previously described procedure.²⁻⁴ The resulting NPs were precipitated and washed twice with ethanol. Amino-functionalized SiO₂ NPs without pores were, then, prepared by dispersing the SiO₂ NPs in 50 ml ethanol, followed by the addition of aminopropyltriethoxysilane (APTES, 0.02 M) and the application of vigorous stirring for 30 minutes. The amino modified SiO₂ NPs were washed and centrifuged twice with ethanol to remove the excess APTES.

Loading and release of the dyes

Loading and release of rhodamine B were achieved as follows: amino-modified SiO₂ NPs (15 mg) were reacted with N-carboxyethyl-nitrospiropyran (SP-COOH, 1 mM) using N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide (EDC, 5 mM) and N-hydroxysuccinimide (NHS, 5 mM) as crosslinking agents in HEPES buffer (10 mM, pH 7.0) for 2 hours. It should be noted that SP-COOH was initially dissolved in dimethyl sulfoxide (DMSO, 200 μM). The SiO₂ NPs were precipitated by

centrifugation at 10000 rpm for 2 minutes, and resuspended in HEPES solution. The solution was, then, irradiated with UV light ($\lambda = 365\text{ nm}$) for 10 minutes, and incubated with rhodamine B (100 μM) in the dark for 2 hours. The entrapment of rhodamine B in the pores was carried out by irradiating the NPs with visible light ($\lambda > 495\text{ nm}$) for 8 minutes. The solution was, then, washed at least six times with HEPES buffer, until a low fluorescence background of the rhodamine B was achieved. To release rhodamine B, the sample was irradiated with UV light ($\lambda = 365\text{ nm}$) for 10 minutes, kept in the dark for an additional 10 minutes, followed by measuring the fluorescence of the solution after centrifugal separation.

Loading and release of eosin Y were achieved as follows: SiO_2 NPs (15 mg) were reacted with 2,3-dichloro-1,4-naphthoquinone (1 mM) for 2 hours at 50 °C, and the resulting, quinone-modified SiO_2 NPs were reacted with ascorbic acid (1 mM) for 3 minutes. The SiO_2 NPs were centrifuged, resuspended in HEPES, and incubated with eosin Y (1 mM) for 2 hours. The closed state of the pores was achieved by incubation of the eosin Y-loaded SiO_2 NPs with $\text{Fe}(\text{CN})_6^{3-}$ (1 mM) for 10 minutes. The NPs were, then, precipitated, and washed with methyl viologen (MV^{2+}) (0.5 mM) for 5 minutes and washed five additional times with the buffer, until a low fluorescence background of the dye was achieved. For the release of eosin Y, the SiO_2 NPs were reacted with ascorbic acid (1 mM) for 3 minutes followed by precipitation, and then re-dispersion in HEPES buffer for 10 minutes. Loading and release of doxorubicin was achieved similarly, using reduced β -nicotinamide adenine dinucleotide (NADH) as reducing agent. (Washing the samples with MV^{2+} was not included.)

The loading of the pores with rhodamine B or eosin Y and the extent of release of the respective dyes from the pores was determined by the following procedure. The mesoporous SiO₂ NPs (15 mg) were introduced into 1 ml of 100 μM rhodamine B or 1 mM eosin Y, and were incubated for two hours. The SiO₂ NPs were separated and by recording the fluorescence spectra of the solution before and after addition of the SiO₂ NPs, and using appropriate fluorescence calibration curves, the total amounts of the dyes associated with the NPs were evaluated (dyes bound to pores and non-pore areas). By measuring the cumulative fluorescence spectra of the respective solutions obtained in the washing steps, and using the respective fluorescence calibration curves, the remaining total loadings of the mesoporous SiO₂ NPs were determined. The saturation fluorescence generated in the solutions upon photonic or redox opening of the pores and the respective fluorescence calibration curves, were used to evaluate the content of dyes released from the pores.

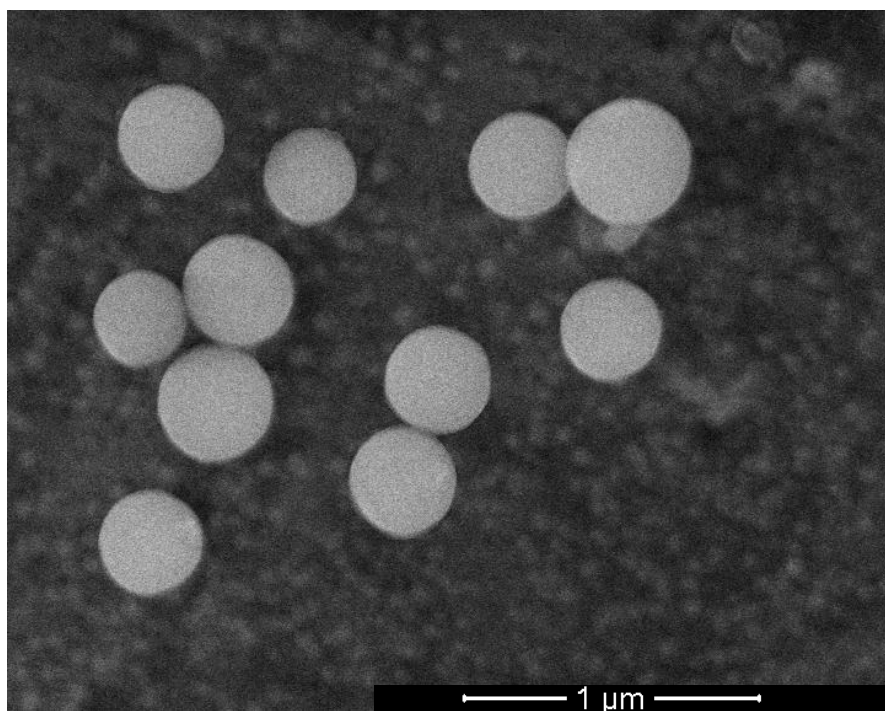


Figure S1. SEM image of solid mesoporous SiO₂ nanoparticles.

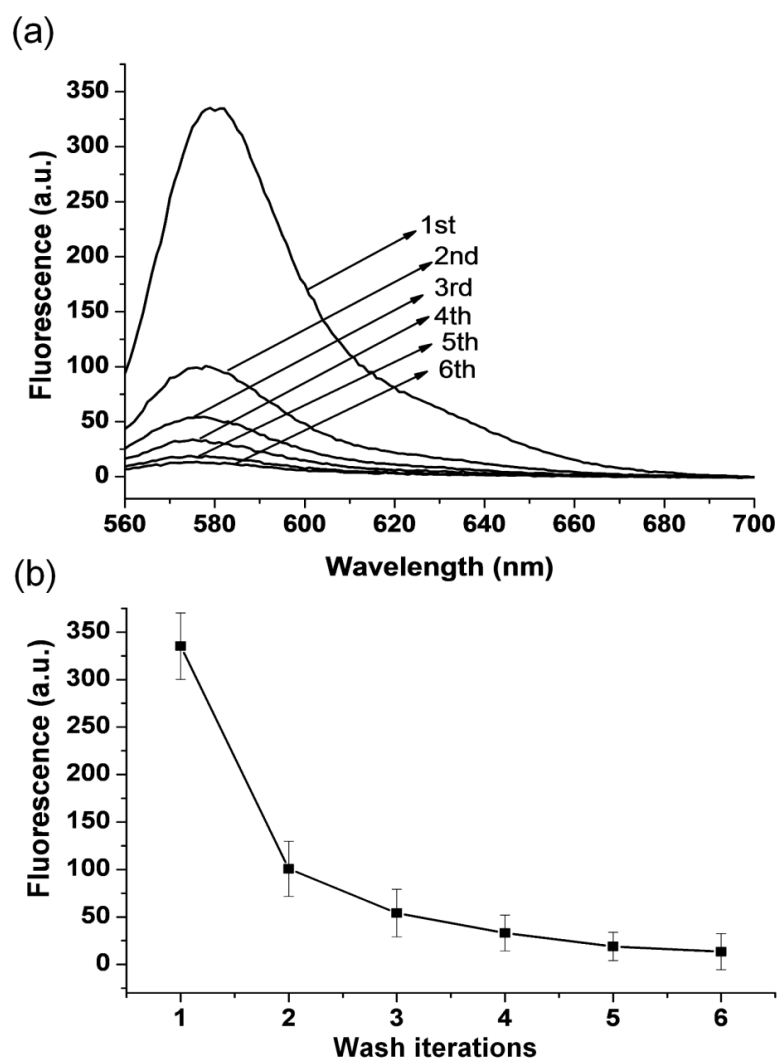


Figure S2. a) Fluorescence spectra of rhodamine B that present the preparation of the rhodamine B loaded pores of the mesoporous SiO₂ NPs. The spectra correspond to the fluorescence of rhodamine B, released from the non-pore, exterior domains of the SiO₂ NPs upon related washing steps with HEPES buffer (10 mM, pH 7.0). b) Fluorescence intensities of rhodamine B removed from exterior domains, as a function of the number of washing steps. The results show that after six washing steps the dye associated with non-pore, exterior domains was almost fully washed off.

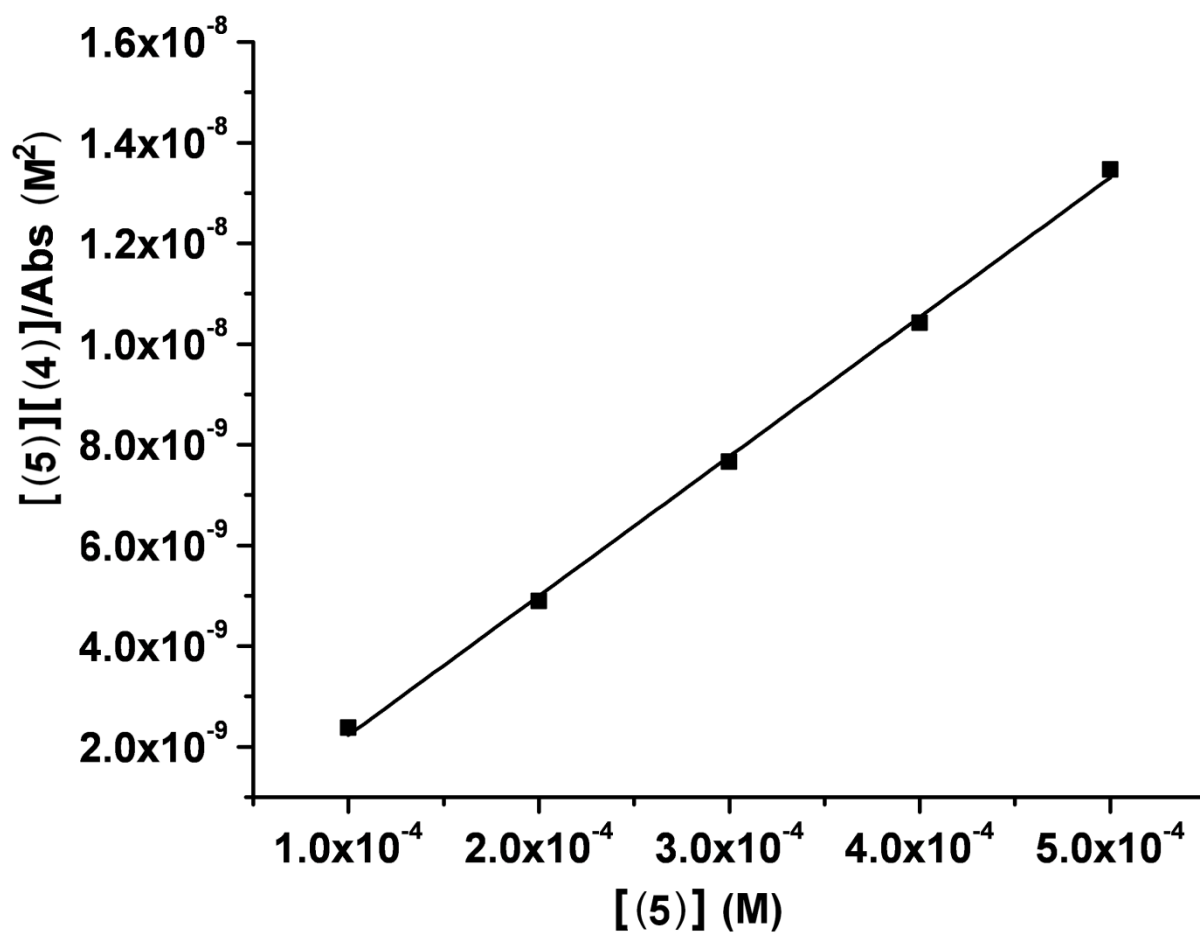


Figure S3. The determination of the association constant between 2-chloro-3-[(2-hydroxyethyl)amino]-1,4-naphthoquinone, (5), and eosin Y, (4), according to the Scott method.

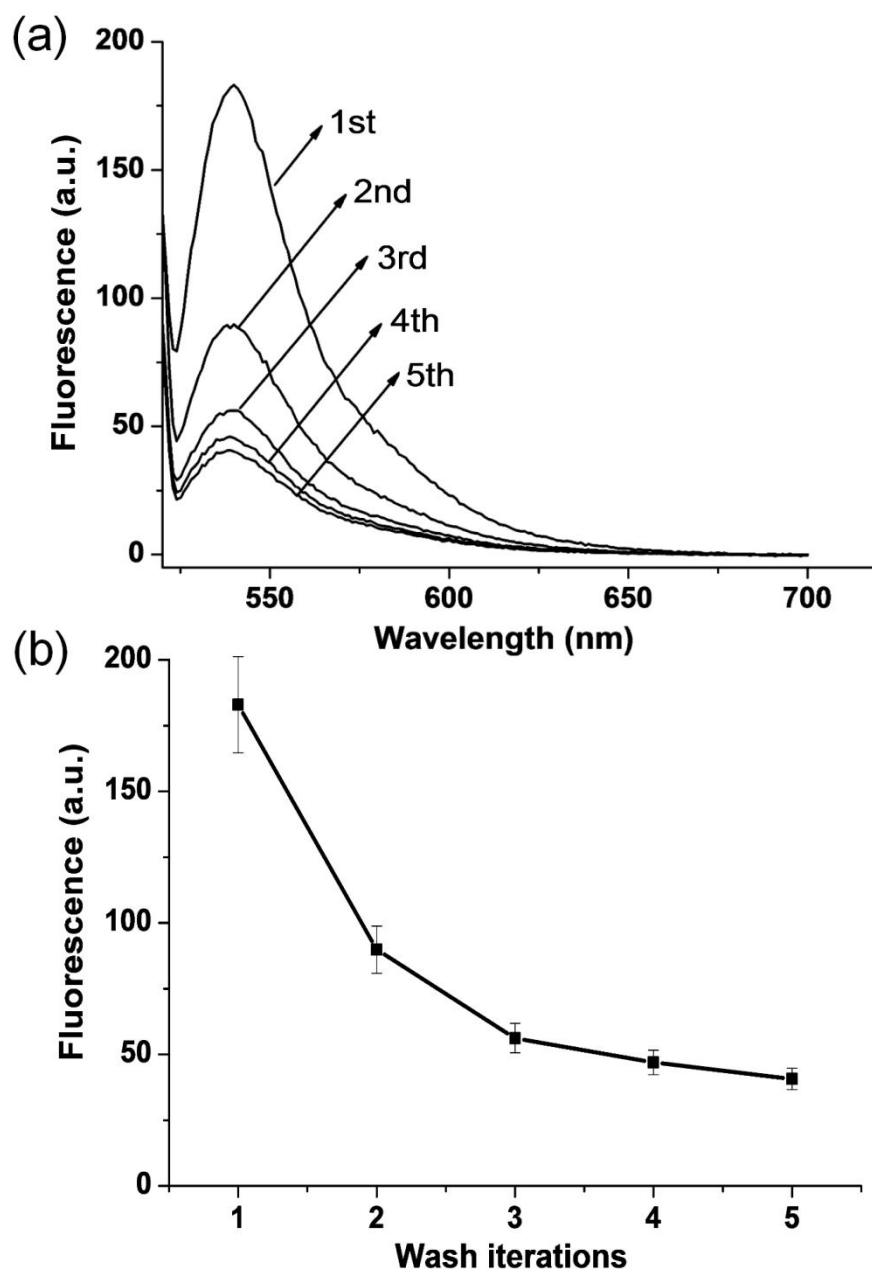


Figure S4. a) Fluorescence spectra of eosin Y that present the preparation of the eosin Y loaded pores of the mesoporous SiO₂ NPs. In the first step most of the eosin Y associated with non-porous domains was washed off using a solution of methyl viologen (0.5 mM). In the second step, the residual eosin Y associated with non-pore, exterior domains was washed off with a HEPES buffer solution (10 mM, pH 7.0). The fluorescence spectra in (a) correspond to the fluorescence of the buffer washed-off

eosin Y, using several washing steps, (1) to (5). It should be noted that the MV^{2+} -removal of eosin Y from surface, non-porous domains could not be followed by fluorescence spectroscopy, due to the quenching of eosin Y by MV^{2+} . This is the reason for adopting a two-step removal of eosin Y from the exterior domain that involved the primary removal of the dye by MV^{2+} , followed by washing the NPs with the buffer solution. The use of the second step ensured that no eosin exists in the exterior domains. b) Fluorescence intensities of eosin Y as a function of the number of washing steps. The decrease in the fluorescence intensities indicates that the amount of eosin Y released in each washing step from the SiO_2 NPs decreases and that after six washing steps eosin Y is almost fully eliminated from non pore, exterior regions.

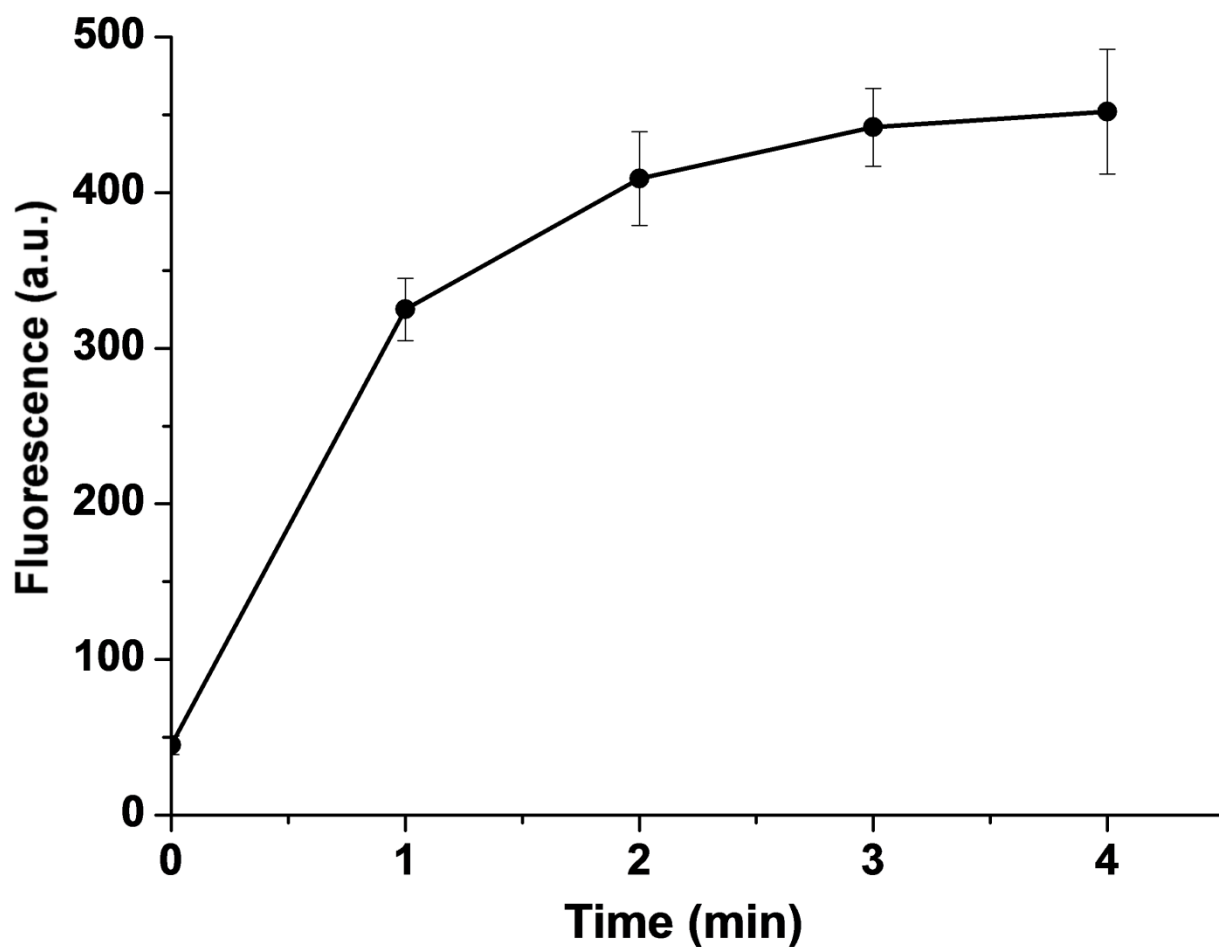


Figure S5. Optimizing the time-interval for the reduction of the (3a)-quinone valves to the (3b)-hydroquinone valves. Curve represents the fluorescence intensity of eosin Y released after a time-interval of 10 minutes of (3a)-modified porous SiO₂ NPs systems, treated for different time-intervals with ascorbic acid (1 mM). The curve implies that treatment of the NPs with ascorbic acid for three minutes leads to a fully open pore structure.

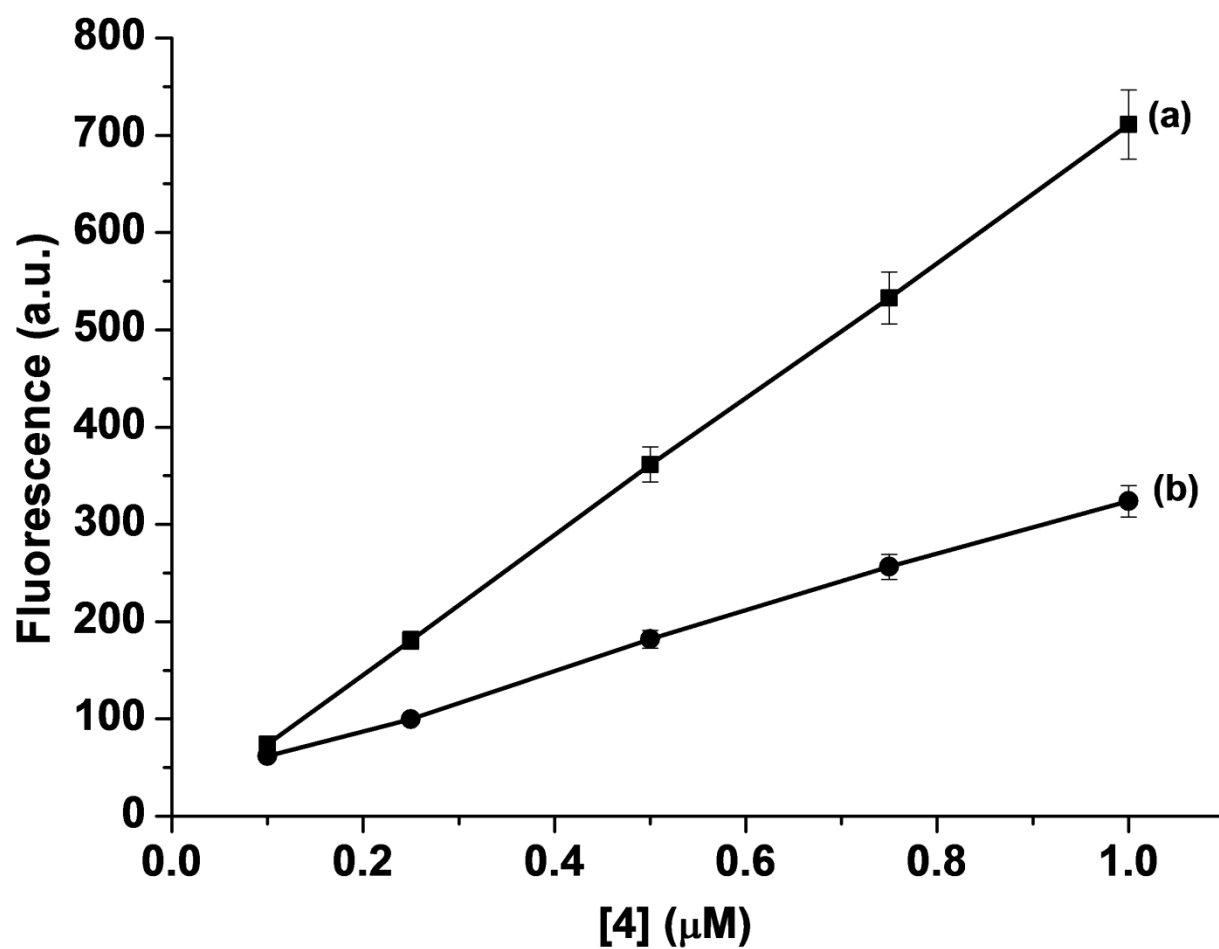


Figure S6. Calibration curves corresponding to the fluorescence intensities of different concentrations of eosin Y in: (a) A pure HEPES solution (0.01 M, pH 7); (b) In a HEPES solution (0.01 M, pH 7), containing ascorbic acid (1 mM).

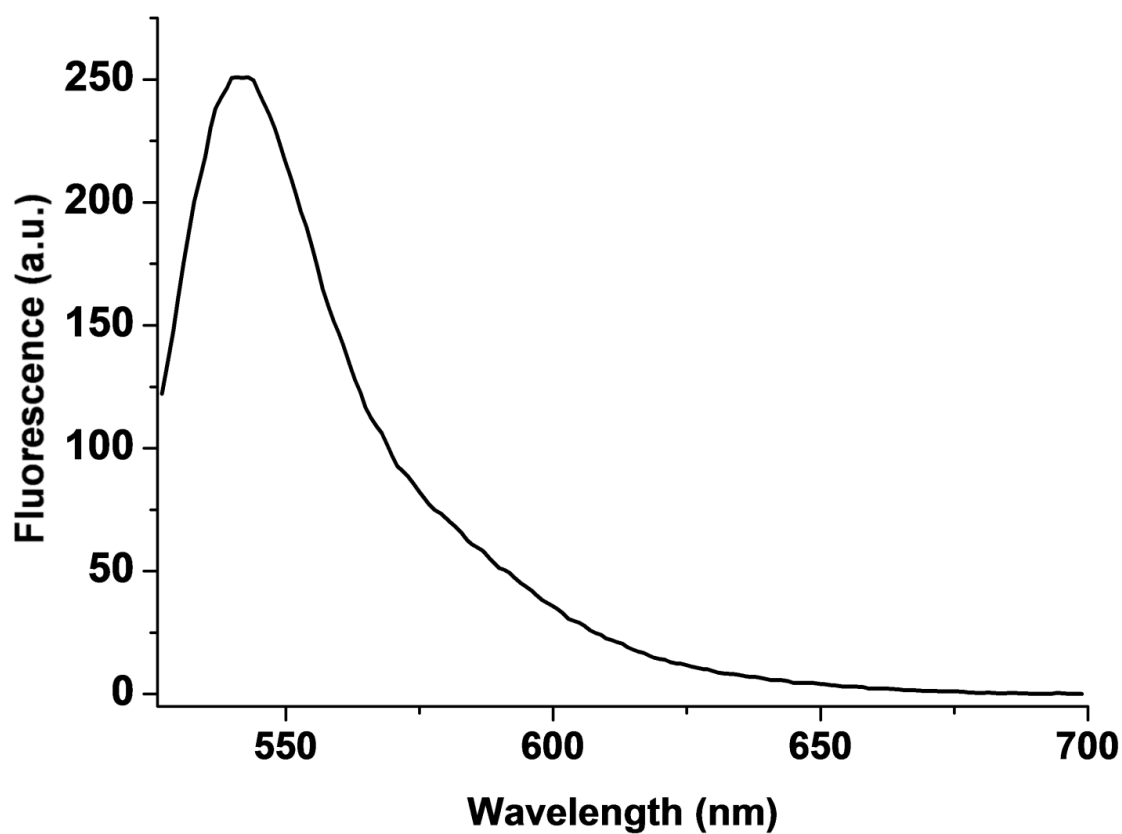


Figure S7. Fluorescence intensity of fully-released eosin Y from the pores of (3b)-functionalized SiO₂ NPs into a HEPES solution (0.01 M, pH 7) that includes ascorbic acid (1 mM).

Imaging of fluorescent MP-SiO₂ NPs incorporated into normal and malignant human cells.

The respective cells were incubated with the fluorescein-functionalized MP-SiO₂ NPs for a time-interval of six hours. The anti-fluorescein antibodies were added to the cell culture to quench the fluorescence of the fluorescent NPs outside the cells. The cells were imaged by optical microscopy and parallel epi-fluorescence microscopy. Figure S8 depicts the optical microscopy image (a) of the fluorescent NPs incorporated HepG2 cells, the epi-fluorescent image (b) and the overlay image (c).

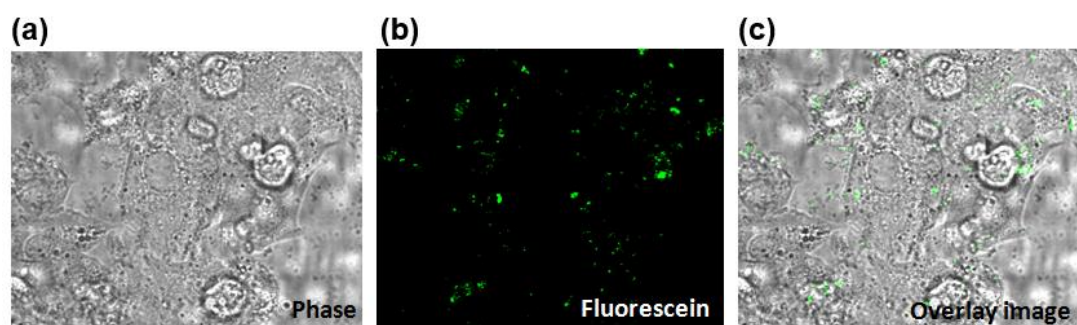


Figure S8. Microscopy images of the HepG2 cells with incorporated fluoresceine-functionalized MP-SiO₂ NPs: (a) optical image; (b) epi-fluorescence image and (c) overlay image.

Monitoring the cytotoxicity effects of different composites of MP-SiO₂ NPs on human normal (HEK-293) cells and breast cancer (MDA-231) cells.

Human normal (HEK -293) cells and breast cancer (MDA-231) malignant cells were planted at a density of 2.5×10^5 cells/well in 24-well plates. After overnight, cells were incubated with samples of MP-SiO₂ (150 µg/ml) solubilized in the appropriate growth medium, for six hours. After intensive washing of the cells with growth medium, cell viability was determined with the fluorescent redox probe, Alamar blue^{5,6}. The fluorescence of Alamar blue was measured with a plate-reader (Tecan Safire) after 1 hour incubation at 37°C (Exc:530-560nm; Em: 590nm).

References:

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