

## ***Supporting Information***

### **DNA-Templated *in situ* Growth of Silver Nanoparticles on Mesoporous Silica Nanospheres for Smart Intracellular GSH-Controlled Delivery**

Changhui Liu,<sup>a,b</sup> Zhihe Qing,<sup>c</sup> Jing Zheng,<sup>a</sup> Li Deng,<sup>a</sup> Cheng Ma,<sup>a</sup> Jishan Li,<sup>a</sup>  
Yinhui Li,<sup>a</sup> Sheng Yang,<sup>a</sup> Jinfeng Yang,<sup>d</sup> Jing Wang,<sup>d</sup> Weihong Tan<sup>a,e</sup> and  
Ronghua Yang,<sup>\*a,c</sup>

*<sup>a</sup>State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and  
Chemical Engineering, Collaborative Innovation Center for Chemistry and Molecular  
Medicine,*

*Hunan University, Changsha, 410082, P. R. China.*

*<sup>b</sup>Department of Chemistry and Environmental Engineering,*

*Hunan City University, Yiyang, 413000, P. R. China*

*<sup>c</sup>School of Chemistry and Biological Engineering,*

*Changsha University of Science and Technology, Changsha, 410004, P. R.China;*

*<sup>d</sup>The Affiliated Cancer Hospital of Xiangya School of Medicine,*

*Central South University, Changsha, 410011, P. R. China.*

*<sup>e</sup>Center for Research at the Bio/Nano Interface, Department of Chemistry and Department of  
Physiology and Functional Genomics,*

*University of Florida, Gainesville, Florida, 32611-7200, USA.*

## EXPERIMENTAL SECTION

### Materials and Reagents:

Tetraethylorthosilicate (TEOS), *N*-cetyltrimethylammonium bromide (CTAB), sodium hydroxide (NaOH), 3-isocyanatopropyltriethoxysilane (3-ICP), silver nitrate (AgNO<sub>3</sub>), 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES), rhodamine 6G (Rh6G), and (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Alfa Aesar Chemical Ltd. Sartorius ultrapure water (18.2 MΩ; Millipore Co., USA) was used in all experiments and the preparation of all buffers. All chemicals were of analytical grade and used as received without further purification. The oligonucleotides were synthesized by TaKaRa Biotechnology Co., Ltd., (Dalian, China). The human hepatoblastoma cell line (HepG2) and cervical carcinoma cancer cell line (HeLa) were obtained by the Cell Center of our lab.

### Methods:

**Synthesis of MCM-41-type MSNs:** *N*-cetyltrimethylammonium bromide (CTAB, 0.5 g, 1.37 mmol) and sodium hydroxide aqueous solution (1.75 mL, 2.0 M) were first dissolved in 240 mL of nanopure water. After the solution temperature was adjusted to 353K, tetraethoxysilane (TEOS, 2.50 mL, 12.8 mmol) was added dropwise to the surfactant solution under vigorous stirring. The solution was allowed to react for 2 h to give rise to a white precipitate, following by centrifugation, washing with deionized water and ethanol, and drying in air to yield the as-synthesized mesoporous silica nanoparticles (MSNs). To remove the surfactant template (CTAB), 0.50 g of the as-synthesized MSN were refluxed for 16 h in a mixture of 2.50 mL of HCl (37.4 wt % in water) and 50.0 mL of methanol, following by filter and extensively washing with deionized water and methanol. The surfactant-free MSNs were placed under high vacuum to remove the remaining solvent from the channel of mesoporous silica.

**Synthesis of DNA-modified MSNs:** 1.00 g of the obtained MSNs were refluxed for 20 h in 80.0 mL of anhydrous toluene with 0.25 mL (0.25 g, 1.00 mmol) of 3-isocyanatopropyltriethoxysilane to yield 3-isocyanatopropyl-modified MSNs (MSNs-ICP). After removal of toluene by centrifugation, the purified MSNs-ICP (100 mg) was redispersed in 2.0 mL of deionized water. 200  $\mu$ M of the amino-modified oligonucleotide were then added. The amino groups of DNA (P1, 5'-NH<sub>2</sub>-ACCCCTACCCCTACCCC-TACCCCTACC-3') were allowed to react with the ICP functional groups presented on the surface of MSN overnight at room temperature to obtain the DNA-functionalized MSN (MSN-P1).

**Cargo loading AgNPs-capped MSNs:** The purified MSN-P1 (50 mg) was stirred in a solution of guest molecules (Rh6G) in 3.0 mL PBS (10 mM, pH 7.4) for 24 h in dark. Then, the mixture was dispersed in 50.0 mL PBS and cooled to 4°C. An aliquot of AgNO<sub>3</sub> solution with different concentration ratios of Ag<sup>+</sup> to P1 was then added and stirred for 30 min. Subsequently, HEPES solution (100 mM, pH 7.4) was added as a reducing agent for 72 h at 40°C in dark (or using NaBH<sub>4</sub> as a reducer overnight at 4°C). Then the drug-loaded AgNPs-capped MSNs (AgNP@MSNs) were obtained by centrifuging and washing extensively with PBS to remove physisorbed, uncapped cargo molecules from the exterior surface of the material. All the washing solutions were collected, and the loading of drug was calculated from the difference in the concentration of the initial and left guests to be 68.7  $\mu$ mol/g SiO<sub>2</sub>.

**In vitro Release Analysis of AgNPs@MSNs:** 1.0 mg of Rh6G-loaded AgNP-2@MSNs was placed in the bottom of a cuvette. 1.0 mL 10% FBS containing different concentrations of GSH triggers was carefully added to the cuvette in a dropwise fashion in order to prevent the nanoparticles from dispersing into the solution. The released Rh6G molecules were determined by fluorescence spectra (exc. at 520 nm; em. at 550 nm). The fluorescence intensity at the emission maximum of the Rh6G molecules was plotted as a function of time

in order to generate a release profile. The amount of released Rh6G was measured by a fluorescence spectrophotometer, thus enabling quantitative comparison of the release efficiency. The weight percentage (wt %) was calculated as the released quantity of cargo divided by the total quantity of loaded nanoparticles.

**Cell Viability Assay:** The cytotoxicity of AgNP-2@MSNs and their released products were investigated by (3-(4-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Typically,  $2 \times 10^5$  cells/well was seeded into a 96-well plate overnight before the cytotoxicity studies. Subsequently, the medium was replaced with fresh medium containing AgNP-2@MSNs with or without 5.0 mM GSH-OEt for 24 h. Medium containing remaining materials was removed by gently rinsing the cultures twice, and fresh medium containing 0.5 mg/mL MTT was added. After 3 h, 150  $\mu$ L of DMSO were added into the well in order to dissolve the insoluble purple formazan. The absorbance was measured by a multidetection microplate reader (Bio-Rad ELISA reader, Hercules, CA). Cells incubated without any materials were used as controls, and the viabilities of these cells were set as 100%. Each concentration was tested at least three times.

**Intracellular Release studies:** For CLSM observations, HeLa cells and HepG2 cells were seeded in cover glass-bottom dish (35 mm  $\times$  10 mm), and HeLa cells were pretreated with glutathione monoester (GSH-OEt, 5.0 mM) and *N*-methylmaleimide (NMM, 5.0 mM) for 2 h, respectively. Rh6G-loaded AgNP-2@MSNs were then added to the cells at the concentration of 100  $\mu$ g/mL, and the mixture was incubated for 3 h in 5% CO<sub>2</sub> at 37°C. The cells were then washed twice with PBS and finally examined under the confocal laser scanning microscope (Fluo View FV1000, Olympus).

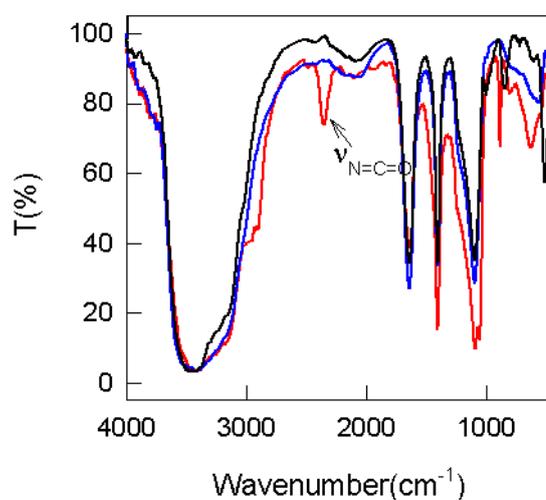
#### **Characterization:**

High-resolution transmission electron microscopy (TEM) images were obtained from a JEOL-3010 using an accelerating voltage of 100 kV. Scanning electron microscopy (SEM)

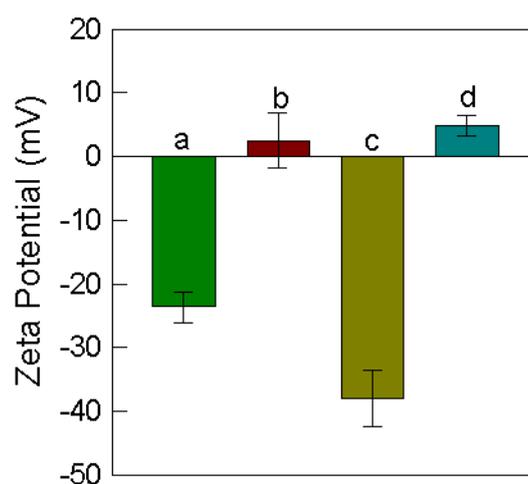
images were obtained on a JEOL JSM-6700F microscope. Zeta potential experiments and DLS measurements were performed at 25 °C using a Malvern ZetaSizer Nanoseries (Nano ZS90). Fourier transform infrared (FT-IR) spectra were obtained from a TENSOR 27 spectrometer (Bruker Instruments Inc., Germany). UV-vis spectra were collected using a Hitachi U-3010 spectrophotometer (Kyoto, Japan). Small-angle powder X-ray diffraction pattern of the MSN materials was obtained in a Scintag XDS-2000 powder diffractometer using CuK $\alpha$  irradiation ( $\lambda=1.54065\text{\AA}$ ). N<sub>2</sub> adsorption-desorption isotherm at 77 K was obtained on a Micromeritics ASAP 2010 sorptometer by static adsorption procedures. Brunauer-Emmett-Teller (BET) surface area was calculated from the linear part of the BET plot according to IUPAC recommendations. Pore size distribution was estimated from the adsorption branches of the isotherms by the Barrett-Joyner-Halenda (BJH) method. The solid-state NMR spectroscopy spectra were obtained at room temperature on a Bruker DSX 300 MHz spectrometer. All fluorescence measurements were performed on a PTI QM4 Fluorescence System (Photo Technology International, Birmingham, NJ) with a temperature control accessory. Confocal laser scanning microscopy (CLSM) images were obtained on a Fluoview FV 500 (Olympus). The MTT assay was obtained in a Benchmark Plus (Bio-Rad Instruments Inc., Japan).

**Table S1.** Properties of the native MSN, MSN-ICP and Rh6G loaded AgNP-2@MSNs.

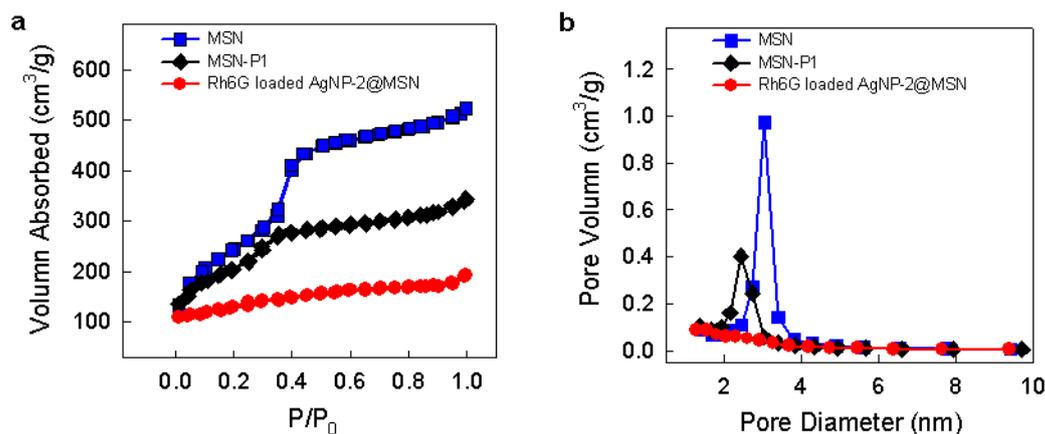
Particles	Surface area (m <sup>2</sup> /g)	Pore Volume (cm <sup>3</sup> /g)	Pore size (nm)	Zeta Potential (mV)
MSN	1057.8	0.832	3.06	-23.6 ±2.4
MSN-P1	527.8	0.455	2.65	-40 ±6.5
Rh6G loaded AgNP-2@MSNs	125.3	/	/	+4.8 ± 2.26



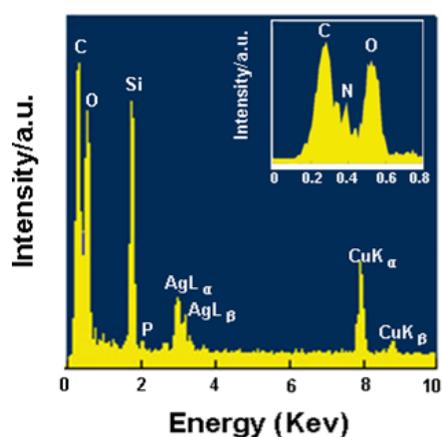
**Figure S1.** FT-IR spectra of the MSN (black), MSN-ICP (red), and MSN-P1 (blue).



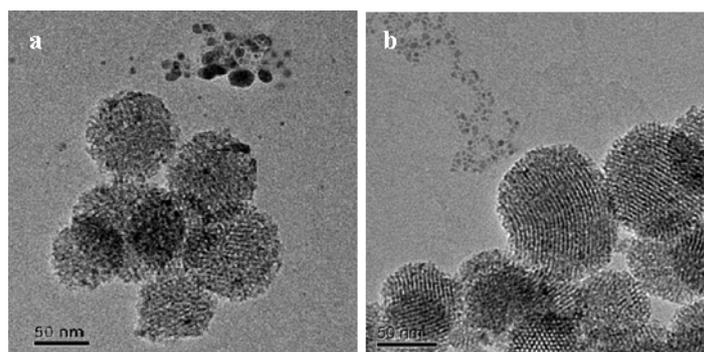
**Figure S2.** Zeta potential values for MSN (a), MSN-ICP (b), MSN-P1 (c) and Rh6G loaded AgNP-2@MSNs (d) in PBS (10 mM, pH 7.4). Error bars indicate s.d. (n = 3).



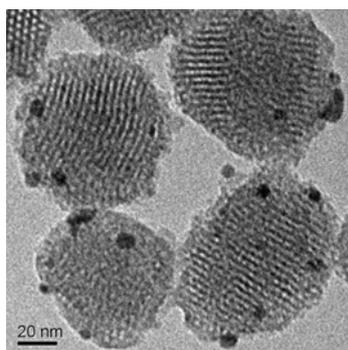
**Figure S3.** Nitrogen adsorption-desorption isotherms (a) and pore size distribution curves (b) of the MSN, MSN-P1 and Rh6G loaded AgNP-2@MSNs. The surface area and pore size of different nanoparticles were determined by Brunauer-Emmett-Teller (BET) and Barrett-Joyner-Halenda (BJH) analysis, respectively. The decrease and then disappearance of the maximum peak in the BJH pore size distribution plot indicate that the mesopores of MSN are capped by DNA and AgNPs.



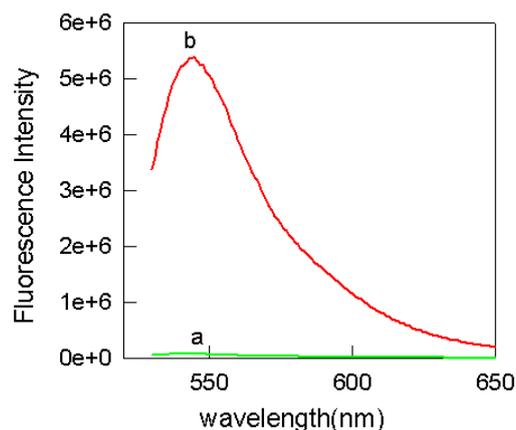
**Figure S4.** EDX spectra of a single Rh6G loaded AgNP-2@MSNs in PBS (10 mM, pH 7.4).



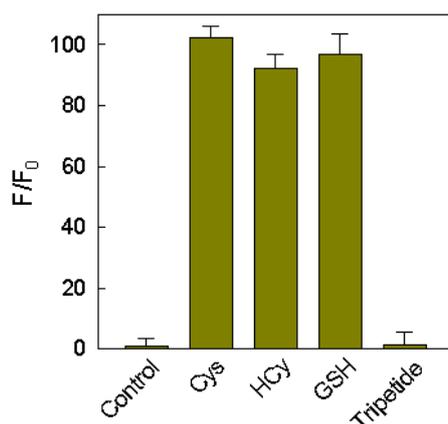
**Figure S5.** TEM micrographs of AgNPs prepared by reducing  $\text{AgNO}_3$  in the presence of MSNs (a) and (MSNs + P1) (b). The reduction products show AgNPs outside of the MSNs surface, indicating that the conjugated DNA strands are required for AgNPs nucleation and growth on the MSN surface.



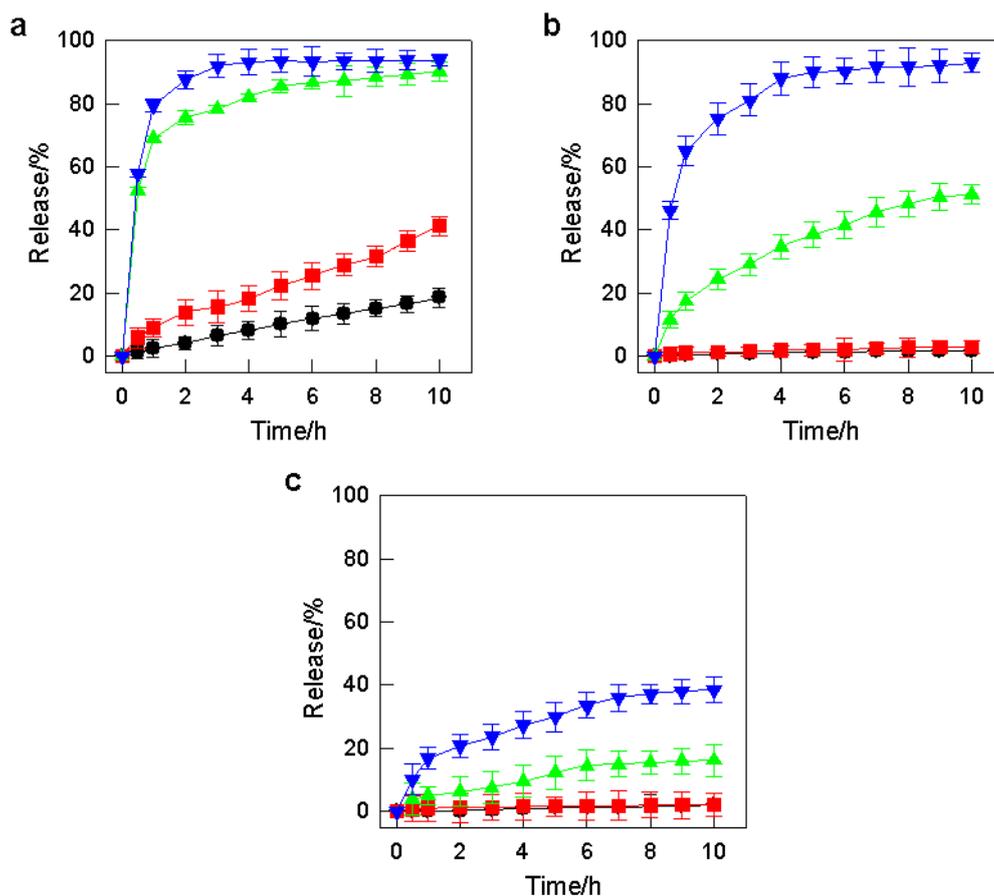
**Figure S6.** TEM micrographs of Rh6G-loaded AgNP-2@MSNs incubated with 5.0 mM GSH for 2 h.



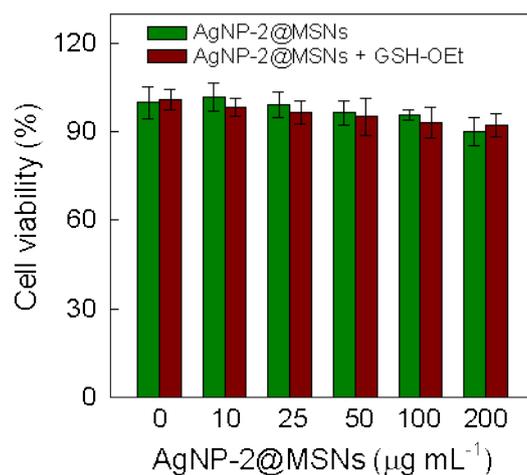
**Figure S7.** Fluorescence emission spectra of Rh6G released from AgNP-2@MSNs ( $\lambda_{\text{ex}} = 520$  nm) before (a) and after (b) treatment with 5.0 mM GSH for 2 h.



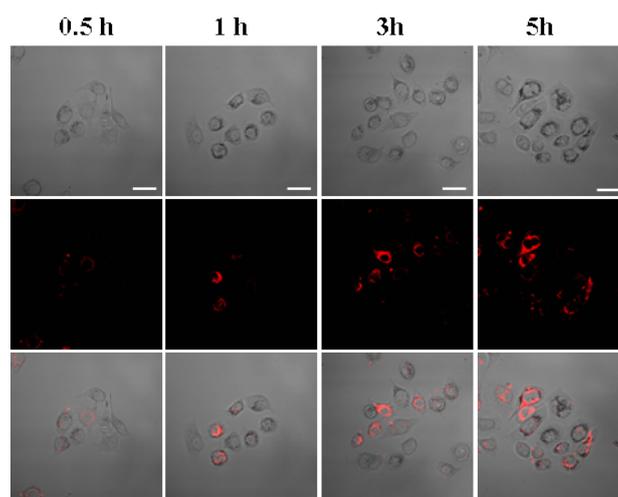
**Figure S8.** Relative fluorescence intensity of Rh6G released from AgNP-2@MSNs in PBS (10 mM, pH 7.4) in the presence of selected substrates (x-axis markers) at 5.0 mM for 2 h.  $F_0$  and  $F$  are the fluorescence intensities of Rh6G loaded AgNP-2@MSNs at 550 nm ( $\lambda_{\text{ex}} = 520$  nm) without and with the following selected substrates: cysteine (Cys), homocysteine (Hcy), glutathione (GSH), and a tripeptide having the same molecular backbone as GSH, but without sulfhydryl group. Error bars indicate s.d. ( $n = 3$ ).



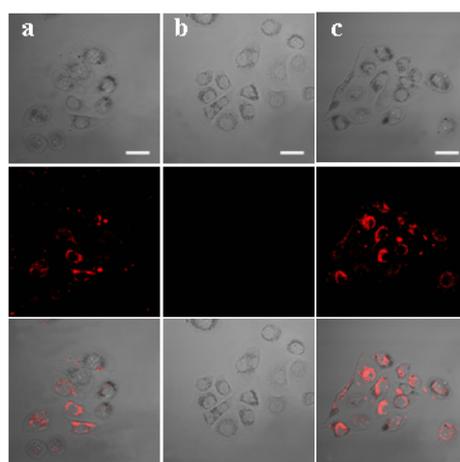
**Figure S9.** Release of Rh6G from AgNP-1@MSNs (a), AgNP-2@MSNs (b) and AgNP-3@MSNs (c) in the presence of GSH: 0 (black); 10  $\mu$ M (red); 1.0 mM (green) and 5.0 mM (blue). Error bars indicate s.d. ( $n = 3$ ).



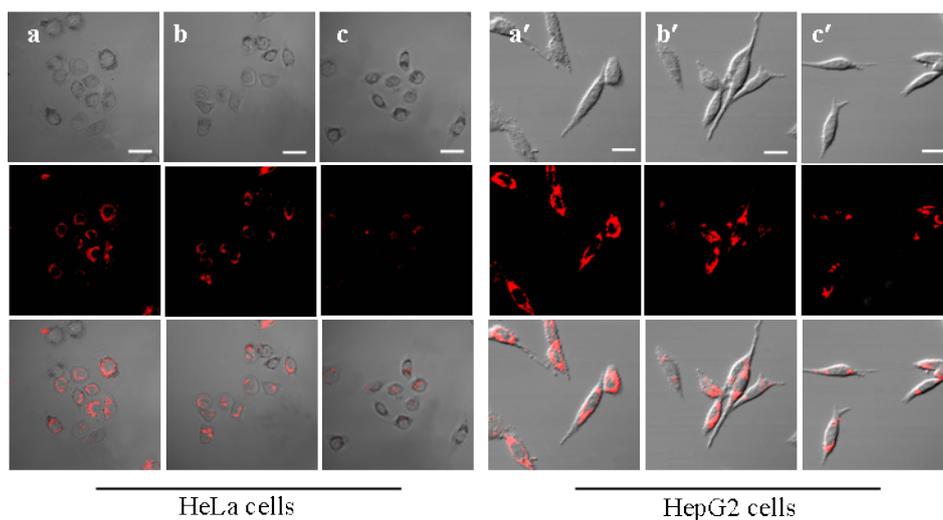
**Figure S10.** *In vitro* cytotoxicity of the blank AgNP-2@MSNs in HeLa cells with and without 5.0 mM GSH-OEt for 24 h.



**Figure S11.** CLSM images of HeLa cells incubated with Rh6G-loaded AgNP-2@MSNs as function of time. Scale bars: 40  $\mu\text{m}$ .



**Figure S12.** Representative CLSM images of HeLa cells incubated with (a), Rh6G-loaded AgNP-2@MSNs; (b), (a) + 5.0 mM NMM and (c), (a) + 5.0 mM GSH-OEt. Scale bars: 40  $\mu\text{m}$ .



**Figure S13.** Intracellular GSH-induced R6G release from Rh6G-loaded AgNP@MSNs in HeLa and HepG2 cells. CLSM images of Rh6G-loaded AgNP-1@MSNs (a and a'), AgNP-2@MSNs (b and b') and AgNP-3@MSNs (c and c') incubated in HeLa cells (a,b and c) or HepG2 cell (a',b' and c') for 2 h. Scale bars: 40  $\mu$ m.