Supplementary information

Temperature-responsive DNA-gated nanocarriers for intracellular controlled release

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Experimental Section

Materials:

DNA oligonucleotides were synthesized and purified by Sangon Biotechnology Co., Ltd (Shanghai, China); 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), doxorubicin (Dox) and Rhodamine B (RhB) were purchased from Sigma Chemical Company; Deoxyribonuclease I (DNase I) was purchased from Solarbio Science and Technology Co., Ltd. (Beijing, China); Cetyltrimethylammonium bromide (CTAB), tetraethyl orthosilicate (TEOS) were purchased from China National Pharmaceutical Group Corporation (Shanghai, China); (3-aminopropyl)triethoxysilane (APTES) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) were purchased from Alfa Aesar Chemical Ltd (tianjin, China). All the chemicals were of analytical grade and used without further purification. Sartorius ultrapure water (18.2 M Ω cm) was used throughout the experiments. The human breast cancer cell line (MCF-7) was purchased from KeyGEN biotechnology Company (Nanjing, China).

Instruments:

High resolution transmission electron microscopy (HRTEM) was carried out on a JEM-2100 electron microscope. N₂ adsorption-desorption isotherms were recorded on a Micromeritics ASAP2020 surface area and porosity analyzer. The samples were degassed at 150 °C for 5 h. The specific surface areas were calculated from the adsorption data in the low pressure range using the BET model and pore size was determined using the Barrett-Joyner-Halenda (BJH) method. X-ray measurements were performed on a Rigaku D/MAX-rA diffractometer using Cu-K α radiation. Thermo-gravimetric analysis were carried out on a TGA/Q50 equipment (N₂, 80 mL/min), with a heating program consisting on a heating ramp of 10 °C per minute from 393 K to 1273 K and an isothermal heating step at this temperature during 30 min. Absorption spectra were measured on a pharmaspec UV-1700 UV-visible spectrophotometer (Shimadzu, Japan). Fluorescence spectra were obtained with FLS-920 Edinburgh Fluorescence Spectrometer with a

Xenon lamp and 1.0 cm quartz cells at the slits of 3.0/3.0 nm. All pH measurements were performed with a pH-3c digital pH-meter (Shanghai LeiCi Device Works, Shanghai, China) with a combined glass-calomel electrode. Absorbance was measured in a microplate reader (RT 6000, Rayto, USA) in the MTT assay. Confocal fluorescence imaging studies were performed with a TCS SP5 confocal laser scanning microscopy (Leica Co., Ltd. Germany) with an objective lens (×40). Flow cytometry was performed using a Beckman Coulter Epics XL (Beckman Coulter, Inc., Brea, CA).

Preparation of MS-NH₂:

Amino modified mesoporous silica was synthesized according to the typical co-condensation method reported previously with some modifications.¹ Cetyltrimethylammonium bromide (CTAB, 0.25 g, 0.69×10^{-3} mol) was first dissolved in 120 mL sartorius ultrapure water. NaOH (2 M, 0.75 mL) was then added to the above solution and the mixture was stirred for 5 min, followed by adjusting the solution temperature to 353 K. After that, TEOS (1.25 mL, 5.6×10^{-3} mol) was first introduced dropwise to the surfactant solution, followed by the dropwise addition of APTES (0.25 mL, 1.07×10^{-3} mol). The mixture was filtered, washed with deionized water and methanol, and then dried in air. To remove the surfactant template (CTAB), 0.5 g as-synthesized amino modified mesoporous silica was refluxed for 24 h in a solution of 3 mL of HCl (37.4%) and 50 mL of methanol followed by extensive washes with deionized water and methanol. Finally, the precipitates were dried for 24 h in vacuum.

Preparation of MS-DNA(RhB) :

1.6 mg MS-NH₂ was added and dispersed in 10 mL RhB solution (0.5 mg/mL). The mixture was stirred for 3 days in darkness to reach the maximum loading. 1 mL above solution was centrifuged (10000 rpm, 10 min) and washed with water twice to remove the RhB molecules absorbed physically on the outer surface of the silica. The precipitates were redispersed in 2 mL MES buffer (10 mM, pH=6.0) for further use. MS-DNA was obtained by coupling the carboxyl group of the oligonucleotide and the amino group on the surface of MS-NH₂ to form the amido bond. Take the 40-base DNA1 for example.(Supporting information, Table 1) 4.7 μ L EDC solution (2.8 mM) was added to 26 μ L of DNA1 (100 μ M) solution and the solution was mixed and reacted for 30 min at room temperature to activate carboxylate groups. Then the mixture was added to 2 mL RhB loaded MS-NH₂ solution with gentle stirring in darkness. The solution was negatively charged and the MS-NH₂ was positively charged, single-stranded DNA could absorb on the surface of MS-NH₂ to cap the pores and prevent the RhB molecules from leaking. After that, the precipitates were centrifuged (10000 rpm,10 min) and washed with PBS buffer (10 mM, pH 7.4) for three times and finally redispersed in 8mL PBS buffer. The final concentration of MS-DNA1 (RhB) is 0.02 mg/mL. As for DNA of 30, 20, 15, 10 bases (DNA2-5) (Supporting information, Table 1), 6.1 μ L, 9.5 μ L, 12.5 μ L, 18.7 μ L EDC solution (2.8 mM) was added to 34 μ L, 53 μ L, 70 μ L, 102 μ L DNA solution (100 μ M), respectively. And then, the other procedures were the same as the methods mentioned above. The final concentrations of prepared MS-DNA(1-5)(RhB) were all 0.02 mg/mL.

Determination of the critical temperature of the DNA valves(DNA1-5):

To determine the critical temperature of the nanocarriers, six samples of each kind of nanocarrier in PBS buffer were heated in water bath with different temperature (Table S2). Each sample was heated for 90 min and the fluorescence intensity (λ_{ex} =532 nm, λ_{em} =575 nm) was measured every 15 min. To get the final fluorescence intensity of the dye completely released from the pores, each sample was heated in the water bath at 90 °C for 2 h. The sample was centrifuged (10000 rpm, 10 min) and the supernate was separated. Then, the precipitates were redispersed in 2 mL PBS buffer. The above procedure was repeated at least twice to ensure the RhB release from the pores completely. The fluorescence intensity of the supernate of dye release from the nanocarrier was calculated as follows: (fluorescence intensity of each sample at different time) / (the final fluorescence intensity of the sample). The experiment was repeated three times and the data are shown as the mean±SD.

Quantitation of RhB loaded into the MS-DNA4(RhB):

To quantify the RhB loaded into the nanocarrier, 2 mL MS-DNA4(RhB) solution was heated in the water bath at 90 °C for 2 h. The sample was centrifuged (10000 rpm, 10 min) and the supernate was separated. Then, the precipitates were redispersed in 2 mL PBS buffer. The above procedure was repeated at least twice to ensure the RhB releasing from the pores completely. The fluorescence intensity (λ_{ex} =532 nm, λ_{em} =575 nm) of the supernate was measured and the concentrations of RhB were determined according to a standard linear calibration curve of RhB (supporting information, Figure S4). The loading content of RhB was calculated to be 0.044 mg RhB per 1 mg MS-NH₂.

Stability of the MS-DNA4(RhB):

To evaluate the stability of the MS-DNA4(RhB), 2 mL as-prepared MS-DNA4(RhB) (0.02 mg/mL) was placed at room temperature in darkness and the fluorescence intensity of the sample was measure at 0, 12, 24, 36, 48 and 60 h, respectively. Finally, the nanocarriers were heating for 90 min in a 39 °C water bath and the fluorescence intensity of the sample was measure. The percentage of dye leaking from the nanocarrier was calculated as the same formula above. The experiment was repeated three times and the data are shown as the mean±SD.

Controlled release of the MS-DNA4(RhB) :

The release amount of RhB under high/low temperature cycles was evaluated to study the reversibility of valve ON/OFF switching. 2 mL MS-DNA4(RhB) (0.02 mg/mL) in PBS buffer was heated in water bath for four ON/OFF cycles. For each cycle, the sample was first heated at 39 °C for 15 min and then the sample was cooled down to room temperature and centrifuged, the fluorescence intensity of the supernate

was measured. Next, the sample was kept at 37 °C for 12 h, and the fluorescence was measured the same as mentioned above. Four cycles were carried out in all. The experiment was repeated three times and the data are shown as the mean±SD.

Nuclease assay:

Two groups of 2 mL MS-DNA4(RhB) (0.02 mg/mL) in PBS buffer (10 mM, pH 7.4, 2.5 mM MgCl₂, and 0.5 mM CaCl₂) were incubated at 37 °C . After allowing the samples to equilibrate (10 min), 1.3 μ L of DNase I in assay buffer (2 U/L) was added to one group. The fluorescence signal of the two groups was monitored for 6 h and was collected at 1h intervals during this period. Then these two groups were heated at 90 °C for 90 min, and the fluorescence was measured after the solution was cooled to room temperature.

Cell culture:

MCF-7 cells were cultured in RPMI-1640 medium with 0.01 mg/mL bovine insulin and maintained at 37 °C in 100% humidified atmosphere containing 5% CO₂ at 37 °C.

Confocal fluorescence imaging:

MCF-7 cells were plated on chamber slides for 24 h. Then MS-DNA4(RhB) (0.04 mg/mL) was delivered into the cells in RPMI-1640 culture medium at 37 °C in 5% CO₂ for 12 h. Cells were then washed three times with PBS buffer to remove the nanocarriers that were not uptake into the cells and 2 mL RPMI-1640 culture medium was added finally. The cells were examined with confocal laser scanning microscopy (CLSM) with 543 nm excitation. Three high/low temperature cycles were performed in the same cells. For each cycle, the cells were heated for 10 min then examined by CLSM with 543 nm excitation at 37 °C for 110 min.

Another group of MCF-7 cells were heated at 39 °C for 10 min only once and cells were examined with confocal laser scanning microscopy (CLSM) with 543 nm excitation. Then the cells were cultured at 37 °C for another 4 h and examined with confocal laser scanning microscopy at 2, 4 h, respectively.

MTT assay:

MCF-7 cells (5000 cells/well) were cultured in 96-well microtiter plates and incubated at 37 °C in 5% $CO_2/95\%$ air for 24 h. MCF-7 cells were incubated with culture medium, MS-DNA4 (0.02 mg/mL, 0.04 mg/mL, 0.08 mg/mL) for 12 and 24 h, respectively. Next, 150 µL MTT solution (0.5 mg/ mL) was added to each well. After 4 h, the remaining MTT solution was removed, and 150 µL of DMSO was added to each well to dissolve the formazan crystals. The absorbance was measured at 490 nm with a RT 6000 microplate reader. The experiment was repeated three times and the data are shown as the mean±SD.

Another group of MCF-7 cells (5000 cells/well) were cultured in 96-well microtiter plates and incubated at 37 °C in 5% CO₂/95% air for 24 h. MCF-7 cells were incubated with culture medium, MS-DNA4(Dox)

(0.04 mg/mL) for 12 h. The cells were then heated at 39 °C for different times. Each heating time lasted for 10 min at 2 h intervals. After heating, the cells were incubated at 37 °C for 24 h. The cells were then treated as mentioned above. Each experiment was repeated at least three times and the data are shown as the mean \pm SD.

Flow cytometry:

Four groups of MCF-7 cells were seeded at 1×10^5 /mL in DMEM with 10% fetal bovine serum. After 48 h, the nanocarrier MS-DNA4(Dox) (0.04mg/mL) was added to incubate with the cells for 12 h. the cells were heated for different times (0, 1, 2, 3, respectively) at 2 h intervals. Each heating time lasted for 10 min. After treatment, the cells were detached from culture flasks using trypsin. Flow cytometry was performed using a Beckman Coulter Epics XL, with excitation at 488 nm.

References:

 J. Kim, H. S. Kim, N. Lee, T. Kim, H. Kim, T. Yu, I. C. Song, W. K. Moon, T. Hyeon, *Angew. Chem. Int. Ed.* 2008, 47, 8438.

Oligonucleotide	Bases	Sequence
DNA1	40	5'-COOH-(CH ₂) ₆ -ACTCCTGGTATGTAGCGCTAACT
		GTAATGGTCACGTCACT-3'
DNA2	30	5'-COOH-(CH ₂) ₆ -ACTCCTGGTATGTAGCGCTAACT
		GTAATGG-3'
DNA3	20	5'-COOH-(CH ₂) ₆ -ACTCCTGGTATGTAGCGCTA-3'
DNA4	15	5'-COOH-(CH ₂) ₆ -ACTCCTGGTATGTAG-3'
DNA5	10	5'-COOH-(CH ₂) ₆ -ACTCCTGGTA-3'

Table S1 The sequences of DNA1-DNA5.

 Table S2 Temperature of the water bath corresponding to different DNA valves.

Oligonucleotide	Setting temperature of the water bath / $^{\circ}C$
DNA1	38, 43, 45, 46, 47, 48
DNA2	37, 41, 42, 43, 44, 45
DNA3	37, 39, 40, 41, 42, 43
DNA4	35, 37, 38, 39, 40, 41
DNA5	35, 36, 37, 38, 39, 40
control	35, 36, 37, 38, 39, 40



Fig. S1 The cargo release profiles of MS-DNA(1-4)(RhB) under different temperature.



Fig. S2 (A) The cargo release profiles of MS-DNA5(RhB) under different temperature. (B) The cargo release profiles of MS(RhB) under different temperature.



Fig. S3 (A) N₂ adsorption-desorption isotherms of MS-NH₂; (B) The pore-size distribution of MS-NH₂.



Fig. S4 The TGA figures of the MSNs (black) and MS-NH₂ (red).



Fig. S5 Standard linear calibration curve of RhB.



Fig. S6 (A) The nuclease stability of MS-DNA4(RhB) in the absence or presence of DNase I. Fluorescence curves of MS-DNA4(RhB) (0.02 mg/mL) in PBS (10 mM) without DNase I (trace a), in the presence of DNase I (trace b). (B) Fluorescence spectra the two samples without (trace a) or with (trace b) DNase I after heating at 39 °C for 90 min.



Fig. S7 The normalized fluorescence intensity of the cells after each high temperature process.



Fig. S8 (A) Confocal fluorescence imaging of MCF-7 cells heated for 10 min and then the cells were cultured at 37 °C for 4 h. Scale bars are 250 μ m. (B) The normalized fluorescence intensity of the cells after each treatment process.



Fig. S9 Flow cytometry data of MS-DNA4(Dox) with different treatment. (A) without heating; (B, C, D) with 1, 2, 3 high/ low temperature cycles, respectively. In each cycle, the cells were first heated for 10 min at 39 °C, and then cultured at 37 °C for 2 h.



Fig. S10 Cell viability of MCF-7 cells incubated with different concentrations of MS-DNA4 (0.02, 0.04 and 0.08 mg/mL) for different time (12 and 24 h).



Fig. S11 Cell viability of MCF-7 cells incubated with MS-DNA4(Dox) (0.04 mg/mL) for different high/low temperature cycles.