Supporting Information

DNA nanomachine-based intelligent logic controlled release systems

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1. Materials and apparatus

Tetraethoxysilane (TEOS, 28%), n-cetyltrimethylammonium bromide (CTAB, ≥99%), ammonium hydroxide solution (25%), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC, 99%), N-Hydroxysuccinimide (NHS, 98%), 3-Aminopropyltriethoxy-silane (APTES, 99%), succinic anhydride (99%), sodium citrate tribasic dihydrate, tannic acid, and Rhodamine B were purchased from Sigma Company. Hydrogen tetrachloroaurate (III) was purchased from Jinke reagent company, China. All DNAs were purchased from TaKaRa Biotech (Dalian, China) and used as received. All buffers were prepared with ultra-pure MilliQ water (resistance > 18 MΩ cm⁻¹).

Scanning electron microscopy (SEM) was performed with a JEOL-6700FE instrument. Transmission electron microscopy (TEM) images were obtained using a Philips CM 200 kV electron microscope. Powder X-ray diffraction (XRD) patterns were collected using a Rigaku D/max 2500 equipped with Cu Kα radiation. UV-Vis spectra were collected using a Hitachi U-4100 spectrophotometer. Fourier transform infrared (FT-IR) spectra were taken on a Bruker-EQUINOX55 spectrometer. All spectra were taken with an instrument resolution of 4 cm⁻¹. All fluorescence spectra were recorded on a Hitachi F-4500 FL Spectrophotometer in PBS buffer. N₂ adsorption-desorption isotherms were obtained at 77 K on a Micromeritics ASAP2020 automated sorption analyzer. The BET model was applied to evaluate the specific surface areas. Pore size and pore volume were determined from the adsorption data by the BJH method. CD spectra were collected on a JASCO J-810 CD spectrometer.

2. DNA Sequences
The underlined sequences in sequences a, c, d represent K⁺, adenosine and cocaine aptamer, respectively. The boldface indicates the mismatch base group, which were designed to ensure the upper part of DNA duplex formed by strands c and d can be dissociated after the addition of target adenosines. DTPA represent the cyclic disulfide-containing phosphate derivative.

3. Preparation of AuNP-DNA conjugates

Gold colloids with mean diameters of 3.6 nm were synthesized by the citrate/tannic acid method. The citrate-stabilized gold colloids were subsequently exchanged with a negatively charged phosphine shell using bis(p-sulfonatophenyl) phenylphosphine dehydrate dipotassium salt. DNA-AuNP conjugates were prepared by mixing AuNPs with the modified ssDNA with a 3:1 mole ratio and incubated for 12 h in 0.5 × TBE buffer (containing 50 mM NaCl). The method used for isolating DNA-AuNP monoconjugates from the reaction mixture is gel electrophoresis (2.0 or 2.5% agarose gel at 10 V/cm, 0.5 × TBE buffer), followed by recovery of the appropriate band. Collected fractions were concentrated in Microcon 10000 MWCO centrifugal filters. The modified AuNP-DNA conjugates were further mixed with MS-DNA to create the controlled release system.

![Representative TEM photograph of AuNPs.](Fig. S1)
4. The Controlled Release Experiment

The controlled release behavior was investigated by monitoring the luminescence intensity of the release system. RhB-loaded MS-AuNPs (10 mg) was placed in a corner of cuvette, which was then carefully filled with PBS solution (10 mM, 0.10 M NaCl, pH = 7.2). For activating the nanomachines, aqueous solution of potassium chloride, adenosine and cocaine (100 µL, 20 mM) were added in the “OR” and “AND” logic gate system, respectively. The amount of released guest molecules from the pores of MS-AuNP was monitored by fluorescence measurements at 570 nm. In order to test the selectivity of the logic system, 100µL of cytidine (20 mM) was added to the system under the same conditions.

5. Characterization of the controlled release system

The scanning electron microscope (SEM), transmission electron microscopy (TEM) images, powder X-ray diffraction (XRD), and N₂ sorption measurement demonstrated a typical hexagonal mesopore channel for MS particles. MS-DNA was confirmed by the characteristic peaks of sugar vibrations at 1460, 1418 and 1386 cm⁻¹, the antisymmetric PO₂⁻ stretching vibrations at 1228 cm⁻¹ and symmetric stretching vibrations at 1086 cm⁻¹ in Fourier transform infrared spectroscopy (FTIR) spectra.

![Representative SEM (a) and TEM (b) photograph of MS materials.](Fig. S2)
**Fig. S3.** XRD pattern of MS as-synthesized, which confirms the presence of a 2d-hexagonal mesostructure with about 3.8 nm lattice spacing.

**Fig. S4.** Nitrogen adsorption-desorption isotherms for the MS material. Inset: Pore size distribution of MS material.

**Fig. S5.** FT-IR spectra of (I) MS particle (II) MS-NH$_2$ (III) MS-COOH and (VI) MS-DNA.

**Fig. S6.** TEM micrographs of MS surface before (a) and after (b) the AuNPs capping. The AuNPs are indicated by small arrows.
Fig. S7. Native polyacrylamide gel analysis of the DNA strands for the construction of “OR” and “AND” logic gates. In Fig. S7 (a), lane 1-3 represent strand b, a, and the reaction products of a and b, respectively; in Fig. S7 (b), lane 1-3 represent strand d, c, and the reaction products of d and c, respectively. The results have proved that the strand a and b, c and d have formed high effective hybridization.

Fig. S8. (a) Demonstration of the structural changes of the DNA nanomachine after the addition of KCl (100 µL, 20 mM). DNA a’ and b’ have the same sequences as that of DNA a and b. A quencher (dabcyl) and a fluorophores (rhodamine green) were labeled at the 5’ ends of DNA a’ and the 3’ ends of DNA b’, respectively. The same experimental conditions were used for this system as that for the controlled release experiment. (b) Fluorescence spectroscopy before (red curve) and after (black curve) the addition of KCl (100 µL, 20 mM), at room temperature (23 ºC) (green curve) and heating to 60 ºC (blue curve).

Fig. S9. CD spectra before (red curve) and after (black curve) the addition of KCl.
As shown in Fig. S8, at the initial state, the fluorophore and quencher are brought to the close proximity to each other (see Fig. S7), resulting in efficient FRET and strong quenching of the rhodamine green fluorescence (high FRET). When K⁺ is added into the system, the G-rich domain of DNA a' forms a folded structure, the fluorophore is separated far away from the quencher. The fluorescence intensity of the solution displays a significant increase (low FRET). As shown in Fig. S9, the CD spectrum of the “OR” DNA logic system before the addition of KCl has a 272 nm positive band and a 247 nm negative band. In contrast, the CD spectrum after the addition of KCl shows a stronger positive band at about 290 nm, with weak negative peaks near 257 nm, which is the characteristic of G-quartet structure consistent with the reported results.[S2-S4] When the temperature was raised to 60 ºC (a value that is higher than the melting point, see Fig. S10) of DNA double strands, the double strands could be dissociated in two separated single-strand DNA.

![Fig. S10](image-url)  
**Fig. S10.** The melting curves of DNA a and b by measuring temperature dependence of UV absorbance at 260 nm.

![Fig. S11](image-url)  
**Fig. S11.** (a) Demonstration of the working principle of the DNA “AND” logic gates. DNA c' and d' have the same sequences as that of DNA c and d. A quencher (dabcyl) and a fluorophores (rhodamine green) were labeled at the 5' ends of DNA c' and the 3' ends of DNA d', respectively. (b) Fluorescence spectroscopy of the system before (black curve) and after the addition of adenosine (red curve), cocaine (blue curve), and the mixture of adenosine and cocaine (green curve). (c) The control experiments, where adenosine and cocaine were respectively
added into the system containing single-strand d'. Black curve: initial state; green curve: after addition of adenosine; and red curve: after addition of cocaine. The same experimental conditions were used for this system as that for the controlled release experiment.

At the initial state (S-IV state), the 3' end of strand c' hybridizes with d' to form the double strand structure (see Fig. S7). The quencher at the 5' end of strand c' remains dangling from the end of the double-strand arm, resulting in relatively lower FRET and partial quenching of the rhodamine green fluorescence (black curve). When target adenosine is solely added into the system, efficient FRET takes place (red curve), indicting that the adenosine could discriminate the active domain of DNA c' to form the aptamer-adenosine complex with hairpin configuration \(^{[S5-S7]}\) and bring the quencher and fluorophore groups together. It is worth noting that the toehold site formed by the adenosine-aptamer interaction (which dissociates a small section of the stiff double strand DNA arm) is pivotal to drive the nanomachine work. When target cocaine is solely added into the system, no obvious fluorescence change was observed (Fig. S11 (b), blue curve), indicating that no the cocaine aptamer complexes were effectively formed by strand d' at this situations (Otherwise, strand d' would form a three-way junction and separate with strand c', resulting in enhanced luminescence).\(^{[S7-S8]}\) When adenosine and cocaine are added into the system at the same time, the fluorescence intensity of the solution displays a significant increase (green curve), indicating that the fluorophore is separated far away from the quencher. The collective actions of adenosine and cocaine have realized the release function.

Fig. S12. Controlled release of RhB from the MS-Au system triggered by K\(^+\) as a function of concentration. The data were obtained at 15 min after the addition of KCl. As displayed in Figure S8, the bioresponsive release system was found to be dependent on the added amount of K\(^+\), and the system exhibited clear increment of release when an increased concentration of K\(^+\) were added from 0 to 30 mM. In the presence of about 20 mM of K\(^+\), the maximum release was observed.
Fig. S13. Analysis of the controlled release behavior of “OR” DNA nanomachine-based switching systems constructed with mono- and multi- DNA modified AuNPs. The systems and experimental condition are (I) single DNA modified AuNPs, no targets; (II) multiple DNA modified AuNPs, no targets; (III) multiple DNA modified AuNPs, KCl (100 µL, 20 mM); (IV) single DNA modified AuNPs, KCl (100 µL, 20 mM); (V) multiple DNA modified AuNPs, heating to 60 ºC, respectively.

HeLa cells were plated on 96 well cell culture clusters and allowed to adhere for 12 h. Cells were incubated solely with RhB-loaded MS particles (100 µg/mL) in cell medium for 12 h at 37 ºC and 5 % CO2. Cell imaging was then carried out with AMG EVOS fl microscopy. Excitation of the HeLa cells incubated with MS was at 531 nm.

For the biocompatibility studies of MS with HeLa and HepG2 cells, cells were plated on 96 well cell culture cluster at a density of 104 cells per well and cultured in 5 % CO2 at 37 ºC for 12 h. Then, MS were added to the media, and the cells were incubated in 5 % CO2 at 37 ºC for 24 h and 72 h. The concentrations of MS were 0.01, 0.1, 1, 10 and 100 µg/mL, respectively. Cell viability was determined by the standard3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay.

Fig. S14 Fluorescence and brightfield images of HeLa cells after incubation with RhB-loaded MS at the concentration of 100 µg/mL and 37 ºC for 12 h: (a) brightfield; (b) the overlay image of a and c; (c) fluorescence (λex = 531 nm).
Fig. S15. Viability test of (a) Hela and (b) HepG2 cells with different concentrations of MS after 24 h and 72 h of incubation. As shown in Fig. S14, even the MS concentration was high up to 100 µg/mL and incubated with Hela and HepG2 cells for 72 h, compared with the control, cells viability was still about 100% (P < 0.05). These indicate that MS is very biocompatible in vitro.

Fig. S16. Cells viability test of a mouse myoblast cell line (C2C12) with different drug released situation of MS after 24 h and 72 h of incubation. As shown in Fig. S15, no MS can reduce C2C12 cells viability after 24 h or 72 h incubation (> 84.7%; P < 0.05).

References