SUPPORTING INFORMATION

Bioresponsive Controlled Release of Cargo with Glucometer Readout from Mesoporous Silica Nanocontainers for Sensing Non-Glucose Targets

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EXPERIMENTAL PROCESS

1. Materials and Reagents

Adenosine 5'-triphosphate (ATP, MW 551.14), cocaine and 3-aminopropyltriethoxysilane (APTES) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Cytosine 5'-triphosphate (CTP), guanosine 5'-triphosphate (GTP) and uridine 5'-triphosphate (UTP) were purchased from Dingguo Biotechnol. Co. Ltd (Beijing, China). Glutaraldehyde, $HAuCl_4 \cdot 4H_4O$ and tetraethoxysilane (TEOS) were purchased from Sinopharm Chem. Re. Co. Ltd (Shanghai, China). Thiolated polyethylene glycol (PEG, MW 2000) was obtained from Nanocs Inc. (New York, NY). All other reagents were of analytical grade and were used without further purification. Ultrapure water obtained from a Millipore water purification system (≥18 MΩ, Milli-Q, Millipore) was used in all runs. Personal glucose meter (PGM) buffer (pH 7.3) was consisted of 72.9 mM Na_2HPO_4, 27.1 mM NaH_2PO_4, 50 mM NaCl and 5 mM $MgCl_2$. Olignonucleotides were purchased from Dingguo Biotechnol. Co. Ltd (Beijing, China), and the sequences were as follows:

ATP aptamer: 5'-ACCTG GGGGA GTATT GCGGA GGAAG GT-3'

DNA1 for ATP: 5'-NH_2-TTTTT *ACCTT CCTCC GCAA*-3'

DNA2 for ATP: 5'-*TACTC CCCCA GGT*TT TTT-SH-3'

Cocaine aptamer: 5'-GACAA GGAAA ATCCT TCAAT GAAGT GGGTC-3'

DNA1 for cocaine: 5'-NH_2-TTTTT *GACCC ACTTC ATTCA*-3'

DNA2 for cocaine: 5'-*AGGAT TTTCC TTGAC* TTTTT-SH-3'

The underlined letters in the DNA1 and DNA2 are complementary to adjacent areas of the corresponding aptamer.

2. Apparatus

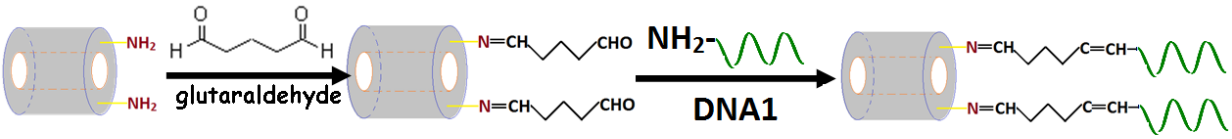
The glucose meter was from Roche Diagnostics GmbH (Mannheim, Germany). The morphology of nanostructures used in this study was characterized by transmission electron microscopy (TEM) on an H-7650 (Hitachi Instrument, Japan) microscope. N_2 adsorption–desorption analysis was measured on a Micromeritics ASAP 2000 instrument (Micromeritics, Norcross, GA, USA). Pore volumes were determined using the adsorbed volume at a relative pressure of 0.99. Multipoint Brunauer–Emmet–Teller (BET) surface area was estimated from the relative pressure range from

0.06 to 0.3. The pore size distributions of the as-prepared samples were analyzed using the Barrett–Joyner–Halenda (BJH) method. Ultraviolet-vis absorption (UV-vis) spectra were recorded with an 1102 UV-vis spectrophotometer (Techcomp, China).

3. DNA1 Conjugation of Mesoporous Silica Nanospheres (DNA1-MSN)

Prior to conjugation with DNA1 strands, the aminated MCM-41 mesoporous silica nanospheres (MSN) were synthesized by using the previous reports.[S1] The BET surface for the MSN sample was about 956 m^2 g^{-1}, which was calculated from N_2 adsorption-desorption isotherm. The mean pore size was 3.2 nm. Meanwhile, the content of nitrogen in the aminated MSN was also determined to be 2.34% by the element analysis.

Next, the aminated MSN was used for the conjugation of DNA1, similar to our previous report.[S2] 10 mg of MSN were initially dispersed into 1-mL ultrapure water containing 200 μL 25 wt % glutaraldehyde, and then stirred for 6 h at room temperature (RT). After separation with centrifugation, the glutaraldehyde-functionalized MSN was dispersed in 500 μL PGM buffer (pH 7.3). Then, 100 μL of DNA1 (5 OD) was added into the prepared-above suspension, and slightly shaken overnight at 4 °C. To reduce the resultant Schiff bases and excess aldehydes, 10 μL of 25 mg mL^{-1} sodium cyanoborohydride was added to the suspension, and incubated for 1 h at 4 °C. Afterwards, the mixture was collected by centrifugation. The obtained pellet (*i.e.* DNA1-conjugated MSN, designated as DNA1-MSN) was re-dispersed into 500 μL PGM buffer (pH 7.3) ($C_{[MSN]} \approx 20$ mg mL^{-1}), and stored at 4 °C until use. Scheme S1 shows the preparation process of DNA1-MSN. By the same token, DNA1 for cocaine was prepared using the similar method.



Scheme S1. Fabrication process of DNA1-functionalized MSN (DNA1-MSN).

4. Preparation of DNA2-Modified Gold Nanoparticles (DNA2-AuNP)

Before labelling, gold nanoparticles (AuNP) with 5 nm in diameter were synthesized by the citrate reduction of $HAuCl_4$, as described in the book.[S3] All glassware used in the following

procedures was cleaned in a bath of $K_2Cr_2O_7$-H_2SO_4, rinsed thoroughly in double distilled water and dried in air. The preparation process is as follows:

1) Prepare 7 mL of a 1 percent $HAuCl_4$ solution in deionized water.
2) Add 6.25 mL of the chloorauric acid solution plus 5.8 mL of 0.1 M K_2CO_3 to 500 mL deionized water. Mix well.
3) In a fume hood, prepare a saturated solution of white phosphorus in diethyl ether, then dilute 1 part of the saturated phosphorus solution with 4 parts of diethyl ether.
4) Add 4.16 mL of the diluted phosphorus solution to the chloroauric acid/carbonate solution with mixing.
5) React at room temperature for 15 minutes.
6) Bring the mixture to a boil and reflux until the color of the suspension turns from brownish to red. This should take no more than about 5 minutes.
7) Cool the sol to room temperature.
8) The pH of the suspension will be around 6. Adjustments to more alkaline conditions for adsorbing macromolecules of higher pI may be done by addition of 0.1 M K_2CO_3 with stirring. After pH adjustment, the gold should be used immediately for complexing with a protein or other macromolecule.

The ζ-potential of the as-prepared gold colloids in the distilled water was -32.1 mV. To eliminate the possible electrostatic interaction between AuNP and MSN, AuNP was further modified by thiolated PEG-2000. 1 mL of citrate-capped AuNP (24 nM) was mixed with 100 μL of PEG-2000 (100 μM). Excess PEG molecules were removed by centrifugation at 13 000*g* for 20 min. Following that, the resulting PEGylated AuNP was re-dispersed in 500 μL distilled water, and the ζ-potential was about -8.73 mV. Afterwards, 100 μL of DNA2 (5 OD) was added into the mixture, and incubated for 12 h at 4 ºC. During this process, DNA2 was conjugated onto the AuNP by Au-S bond (designated as DNA2-AuNP). The DNA2-AuNP was obtained by centrifugation, and dispersed into 500-μL PGM buffer (pH 7.3) for further usage ($C_{[AuNP]} \approx 48$ nM). The ζ-potential of the obtained DNA2-AuNP was -9.76 mV.

5. Loading of Glucose, Preparation of Sensing Platform and PGM Measurement

The loading of glucose solution into the mesoporous silica nanospheres was prepared consulting to the literature.[S4] The positioning of hydroxyl groups around the pyranose rings allows a significant interaction with the curved surface of the silica pore leading to highly localized sugar molecules.[S4] Briefly, 100 μL of the prepared DNA1-MSN ($C_{[MSN]} \approx 20$ mg mL^{-1}) was initially diluted into 500 μL pH 7.3 PGM buffer containing 20 mg glucose, and then the mixture was gently stirred 24 h at 4 ºC. During this process, partial glucose molecules entered into the pores of MSN.[S5] After that, the suspension was centrifuged and washed to remove excess glucose molecules. The obtained DNA1-MSN loading with glucose was dispersed into 500 μL of PGM buffer, pH 7.3. Afterwards, 100 μL of the prepared-above DNA2-AuNP colloids ($C_{[AuNP]} \approx 48$ nM) and 50 μL of the aptamer (5 OD) were added into the glucose-loaded DNA1-MSN mixture, and incubated for 12 h at 4 ºC. During this process, the immobilized DNA1 on the MSN and the labelled DNA2 on the AuNP hybridized with the aptamer to yield a three-stranded complex (designated as MSN-AuNP). The formed DNA complex accompanying with gold nanoparticles was coated on the pores, and gated the glucose molecules inside.

Next, 10 μL of the prepared-above MSN-AuNP suspension was initially injected into 200-μL PCR tube, and ATP standards/samples with various concentrations were added into the tube. The tubes were shaken occasionally during the reaction at RT. Upon addition of target ATP, aptamers specifically and preferentially bound the targets to form the target-aptamer complexes, resulting in the separation of DNA2-AuNP from the DNA1-MSN. In this case, the entrapped glucose molecules were released, which can be quantitative readout by using a simple, low-cost, user-friendly, and portable PGM without the need of sample separation and washing. After incubation for 6 h, a 3-μL aliquot of the supernatant was removed for glucose measurement by using the commercialized Roche PGM. The obtained PGM signal was registered as the sensing signal relative to different-concentration target ATP. All measurements were carried out at room temperature (25 ± 1.0 ºC). All data were obtained with three measurements each in parallel.

The control experiments were carried out by addition of CTP, UTP and GTP to the MSA-AuNP system, respectively.

PARTIAL RESULTS AND DISCUSSION

6. Characterization of DNA1-MSN and DNA2-AuNP

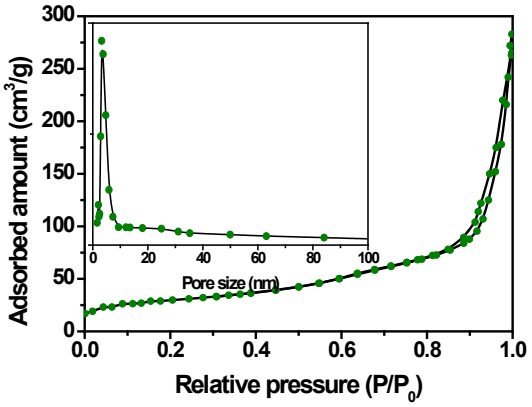


Figure S1. N_2 adsorption-desorption isotherm at 78 K for the aminated MSN sample (inset: Pore size distribution).

As seen from Figure S1, The nitrogen adsorption–desorption isotherm of the aminated MSN exhibits a type IV isotherm, characteristic of mesoporous materials. In the range of 0.7 – 1.0 Pa, step like curves were due to capillary condensation taking place in porous material. The BET surface area was 956 m^2 g^{-1}. A narrow pore size distribution curve showed that pore size with BJH diameter of the most probable distribution was 3.2 nm (inset of Figure S1).

The gas adsorption technique may be used to measure the specific surface area and pore size distribution of powdered or solid materials. The dry sample is usually evacuated of all gas and cooled to a temperature of 77 K, the temperature of liquid nitrogen. At this temperature inert gases such as nitrogen, argon and krypton will physically adsorb on the surface of the sample. This adsorption process can be considered to be a reversible condensation or layering of molecules on the sample surface during which heat is evolved. Nitrogen gas is ideal for measuring surface area and pore size distribution.

An adsorption isotherm (one temperature) is usually recorded as volume of gas adsorbed (cc/g @ STP) versus relative pressure (i.e., sample pressure / saturation vapor pressure). Using relative pressure to construct the isotherm eliminates changes in pressure from small changes in temperature. A small change in temperature changes the saturation vapor pressure considerably. For example, 0.1

K increase in temperature changes the saturation pressure of nitrogen from approx. 760 mm Hg to 800 mm Hg. The use of relative pressure is convenient and is scaled from 0 to 1. A relative pressure of 1 represents a completely saturated sample, i.e., all of the available surface structure is filled with liquid-like gas.

By the same token, we also investigated the characteristics of the as-synthesized DNA2-AuNP by using UV-vis absorption spectroscopy (UV 1102, Techcomp, China). As seen from Figure S2, there were two absorption peaks at 260 nm and 518 nm for the prepared DNA2-AuNP, which were attributed to DNA molecules and gold nanoparticles, respectively.

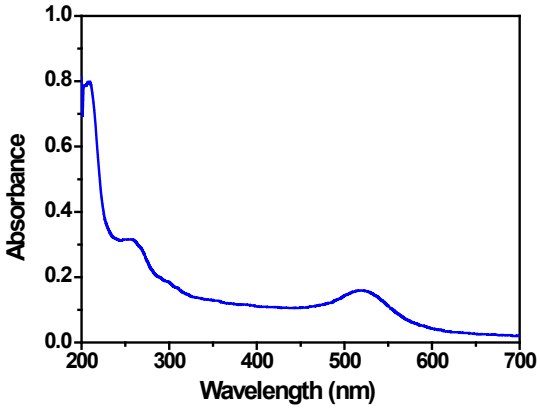


Figure S2. UV-vis absorption spectra of DNA2-AuNP mixture.

7. Loading Efficiency of Glucose

In this work, the PGM signal mainly derived from the entrapped glucose molecules in the pores. Typically, the entrapped glucose amount directly depends on the concentration of glucose in the incubation solution under the fixing other conditions. Meanwhile, glucose molecules might be physically adsorbed on the surface of DNA1-MSN or DNA2-AuNP. Considering this issue, we investigated the effect of various feed glucose masses on the PGM signals by using 0.1 mM ATP as an example. As seen from Figure S3, the PGM signal increased with the increasing glucose, and then tended to level off (≥ 20 mg glucose in 500-μL pH 7.3 PGM buffer containing 100 μL of 20 mg mL^{-1} DNA1-MSN). That is to say, the selected amount of glucose should be more than 20 mg. Taking consideration into the viscosity effect of using high-concentration glucose, 20 mg glucose was preferable.

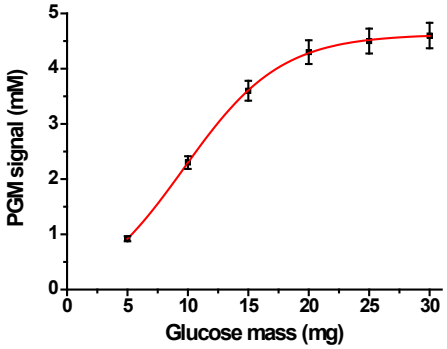


Figure S3. The effect of glucose amount on the PGM signal of the PGM-based method.

To achieve an adequate release of glucose from the pores, the opened number of molecular gate was very important. In this study, the molecular gates were switched on by the target-aptamer reaction. So, we also monitored the effect of incubation time on the PGM signal. As shown in Figure S4, the detectable signals increased with the increment of incubation time, and tended to level off after 6 h. Hence, an incubation time of 6 h was selected for sensitive determination of target ATP at acceptable throughput.

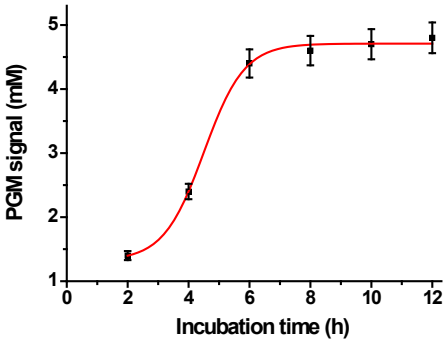


Figure S4. The effect of incubation time for target-aptamer reaction on the PGM signal of the developed system (0.1 mM ATP used in this case).

8. Study of Method Generality for Cocaine

Initially, 10 μL of the prepared-above MSN-AuNP suspension conjugated with cocaine aptamer was injected into 200-μL PCR tube, and then various-concentration cocaine was added into the tube. The tubes were shaken occasionally during the reaction at RT. Upon addition of target cocaine, aptamers specifically and preferentially bind the targets to form target-aptamer complexes, resulting in the separation of functional AuNP from the MSN. In this case, the entrapped glucose molecules

were released, which can be quantitative readout by using a simple, low-cost, user-friendly, and portable PGM without the need of sample separation and washing. After incubation for 6 h, a 3-μL aliquot of the supernatant was removed for glucose measurement by using the commercialized Roche PGM. The obtained PGM signal was registered as the immunosensing signal relative to different-concentration target.

9. Eliminating the Interference of Endogenous Glucose

In certain cases, preexisting endogenous glucose in the sample, *e.g.* human serum, may interfere with the final results. Actually, for small glucose concentrations, the glucose reading before adding into the incubation solution can be subtracted as background. However, for such the sample containing high-concentration glucose, an appreciate dilution should be preferable. To demonstrate this point, cocaine was used as a model. In this experiment, five cocaine standards with various concentrations were determined by using the PGM in the presence of 0, 1 mM and 30 mM glucose, respectively. For containing 1-mM glucose cocaine sample, we directly used the subtracted method. For 30-mM glucose samples, they were initially diluted by using PGM buffer, and then assayed. The real contents of cocaine in these samples were obtained by using the obtained-above calibration curve. The results were compared with the added amount. As indicated from Figure S5, the results of cocaine detection in the samples containing different-level glucose after pretreatments were comparable to those in the glucose-free samples. Therefore, the pretreatment for glucose-containing samples was reliable for successfully eliminating glucose interference.

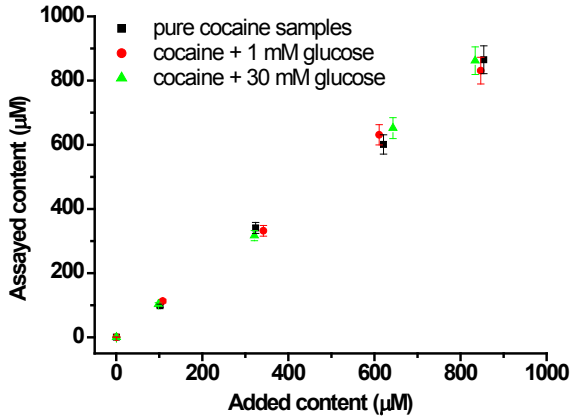


Figure S5. Comparison of the assayed results for cocaine samples in the absence and presence of various-concentration glucose by using the PGM-based method and the added standards.

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