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Supporting Information

DNA-Hybrid-Gated Functional Mesoporous Silica for Sensitive DNA Methyltransferase SERS Detection

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S1 Experimental section

S1.1 Reagents and apparatus

Reagents: All oligonucleotides used in the present study were synthesized by Sangon Biotech Co., Ltd. (Shanghai China) and listed in Table S-1. Methyltransferase of DNA adenine methylation (DNA MTases), endonuclease of Dpn I, S-adenosylmethionine (SAM), and the corresponding buffer solutions were purchased from New England Biolabs (Beijing). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-Hydroxysuccinimide (NHS) was purchased from Sigma-Aldrich. Tetraethylorthosilicate (TEOS), n-cetyltrimethylammoniumbromide (CTABr), 3-aminopropyltriethoxysilane (APTES) were purchased from Sinopharm Chemical Reagent Co., Ltd. (China). $HAuCl_4 \cdot 4H_2O$, trisodium citrate and tri(2-carboxyethyl)phosphine hydrochloride (TCEP, 98%) were ordered from Shanghai Reagent Co., Ltd. (Shanghai, China). The gold chip used for Raman detection was purchased from BioNavis Ltd. All the water used in the work was RNase-free. Phosphate buffered saline (PBS, 0.1 M, pH 7.4) was prepared by mixing the stock solutions of 0.1 M Na_2HPO_4 and 0.1 M NaH_2PO_4 and adjusting the pH with 0.1 M H_3PO_4 or 0.1 M NaOH. Unless otherwise mentioned, ultrapure water was used throughout the experiments. All other reagents employed in this work were analytical

grade and were used without further purification.

Table S1. DNA Sequence Used in This Work.

Note	Sequence
the hairpin DNA	5'-ACT TAT CAG CTT AAG GAT C CCT CAG CAG G ATC CTT AAG CTG ATA AGT-3'
DNA1	5'-CTA AGG ATG CAG CTG AGG GAG TCC TGC TGA GGG A5-COOH-3'
DNA2	5'- CTC CCT CAG CTG CAT CCT TAG TCC TGC TGA GGG A5-COOH-3'
DNA3	5'-NH_2-T_{15}-CAC TGT ATA C-3'
Loading DNA	5'-ATTC AGG GGT GTA TAC AGT G -3'
Rox labelled loading DNA	5'-Rox-ATTC AGG GGT GTA TAC AGT G -3'
DNA-Rox	5'- Rox-GAC GAC GAC T6-SH -3'
Capture DNA	5'-ACC CCT GAA T-T6-SH-3'

Apparatus: Raman measurements were performed on a Renisaw Invia Raman spectrometer (RamLab-010) at an excitation laser of 633 nm. A microscope equipped with a 50 × objective was used to focus the incident excitation laser. The laser power on the sample was 5 mW, and the accumulation time was 10 s. The Raman spectrometer was calibrated with WiRE Raman Software Version 2.0 (Renisaw Ltd.). Transmission electron microscopy (TEM) was measured on a JEOL JEM-2100 instrument. FTIR and UV-Vis spectra were performed on a Nicolet iS10 (Thermo Scientific) and Cary 60 UV-Vis (Agilent Technologies) respectively.

S1.2 Synthesis of 3-aminopropyl functionalized MSNs (NMSNs).

MSNs was synthesized according to the literature by Huangxian Ju's group[1]. CTAB (0.052 g) was dissolved in the mixture of distilled water (25 ml) and NaOH (1 ml, 0.36 M), followed by heating the mixture to 95 ºC. After the temperature was stable, TEOS (2 ml) was added to the mixture slowly. The solution was stirred vigorously for 3 h at 95 ºC. The white product was filtered and washed with water and

ethanol alternatively. Finally the product was dried under vaccum and calcined at 550 ºC to yield the synthesized MSNs.

These MSNs were then functionalized with 3-aminopropyltriethoxysilane (APTES). In brief, MSNs (1 g) was dispersed in ethanol (100 ml), then APTES was added in the solution dropwise. The mixture was stirred for 6 h at 36 ºC. The as-synthesized product was filtered and washed with water/ethanol several times alternatively. Finally the product was dried under vaccum to obtain the synthesized NMSNs.

S1.3 Preparation of MSN-DNA-DNA1/2

The preparation of MSN-DNA-DNA1/2 was performed according to the literature [2]. NMSNs (5 mg) was ultrasonic dispersed in 900 μL PBS buffer (0.1 M, pH7.4) to maximize the dispersion. Next, loading DNA (100 μL, 10^{-4} M) was added following by continuously shaken with 290 rpm at 20 ºC for 24 h to obtain MSN-DNA.

The bio-gate DNA1/2 was prepared right before experiment as follows. The stock solutions of DNA1 and DNA2 were diluted in PBS buffer (pH7.4, $MgCl_2$ 12.5 mM). Then DNA1 (100 μL, 10^{-4} M) and DNA2 (100 μL, 10^{-4} M) were mixed and heated to 95 ºC for 2 min. Therefore the mixture was allowed to cool to room temperature to yield DNA1/2. A series of volume of DNA1/2 were mixed with EDC (100 μL, 10 mg mL^{-1}) and NHS (100 μL, 10mg mL^{-1}) , followed by adding PBS buffer (pH7.4) to make the total volume to 500 μL. The mixture was incubated at 37 ºC for 15 min to active -COOH group modified on the both end of DNA1/2. Then MSN-DNA (500 μL, 5 mg mL^{-1}) was added into the mixture and shaken at 37 ºC for 12 h. The product was washed three times to remove unreacted DNA1/2, and the final product MSN-DNA-DNA1/2 was collected by centrifugation.

S1.4 Synthesis of gold nanoparticles

Gold nanoparticles (Au NPs) were prepared by mixing tetrachloroauric acid ($HAuCl_4$) with trisodium citrate according to the references [3]. In detail, after boiling the $HAuCl_4$ solution (50 mL, 0.01%), trisodium citrate (0.75 mL, 1%) was rapidly added to the solution and stirred for 20 min at the boiling point. The color of the

solution was changed to wine red, which could reveal the formation of Au NPs. The solution was cooled naturally to room temperature with continuous stirring, and was stored in brown glass at 4 °C for further use. The obtained Au NPs were characterized by TEM (Fig. S1).

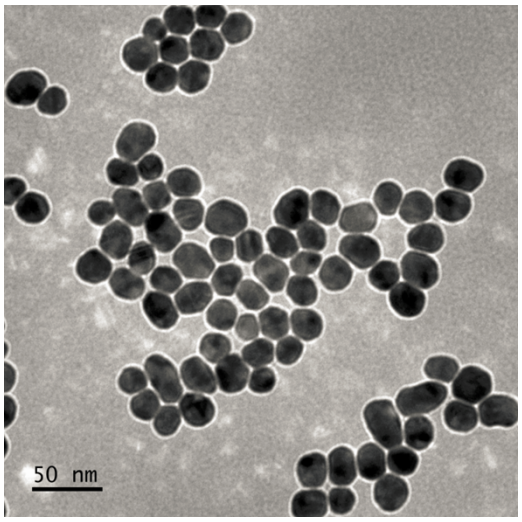
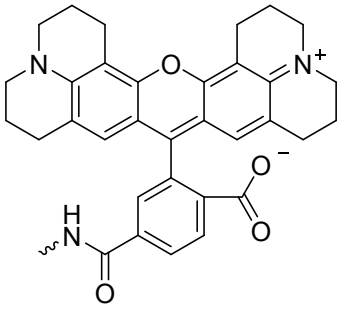


Fig. S1. TEM image of Au NPs synthesized (about 25 nm).

S1.5 Preparation of SERS signal probe

The Au NPs functionalized SERS signal probe was prepared as follows [4]. Briefly, 30 μL of 1×10^{-4} M DNA-Rox (3'-thiol, 5'-Rox) and 10 μL of 1×10^{-5} M capture DNA (3'-thiol) were activated with acetate buffer (pH 6.8) and 10μL of 10 mM TCEP at 37 °C for 1 h. Therefore, 1 mL Au NPs was added and shaken gently at 37 °C for 24 h. Then, the solution was aged by salts (0.05 M NaCl, 200 μL) for 6 h and salts (0.1 M NaCl, 200 μL) for 6 h, respectively. The solution were centrifuged (10000 rpm, 30 min) to collect red precipitate. The obtained red precipitate was washed three times to remove excess reagents. The resulting SERS signal probe was finally dispersed into 1000 μL PBS buffer solution (0.01 M, pH 7.4) and stored at 4 °C.

The structure of Rox:



S1.6 Immobilization of DNA onto MB

Coupling DNA3 onto the surface of MB was performed according to the reference [5]. First, 20 μL suspension of -COOH modified MBs was placed in a 1.5 mL EP tube and separated by magnetic, followed by washing with imidazol-HCl buffer (pH 6.8, 0.1 M, 200 μL) for three times. Then MBs were activated in 200 μL of 0.1 M imidazol-HCl buffer (pH 6.8, containing 0.1 M EDC) at 37 °C for 30 min. Then 20 μL -NH_2 modification DNA3 (1×10^{-4} M) were added to the above MBs and shaken at 37 °C for 12 h. After incubation, the excess DNA was removed by magnetic separation. The resulting DNA3 coupled MBs were rinsed three times with 200 μL of PBS (0.01 M, pH 7.4), and dispersed in 400μL PBS buffer (0.1 M, pH 7.4), stored at 4 °C (noted as DNA-MB).

S1.7 Measurement of the release loading DNA by SERS.

Methylation and Cleavage of Hairpin DNA is carried out according to the literature [6]. The methylation reaction was proceed in 100 μL of reaction mixtures containing various amounts of Dam MTase, 1×10^{-5} M hairpin probe, 1× Dam buffer (50 mM Tris-HCl (pH 7.5), 10 mM NaCl, 10 mM EDTA, 5 mM 2-mercaptoethanol), and 32×10^{-5} M SAM. After methylation, 50 μL 4×NEB buffer 4 (50 mM KAc, 20 mM Tris-Ac, 10 mM $Mg(Ac)_2$,1 mM DTT (pH 7.9)) and 50 μL Dpn I (200 U) for the cleavage reaction were added to the 100 μL methylation products. After the mixture was incubated at 37 °C for 1.5 h, 40 μL Nb.BbvCI (100 U), 30 μL 10×cutsmart buffer, 30 μL PBS buffer (0.1 M, pH 7.4) and 5mg MSN-DNA-DNA1/2 were added. After incubation at 37 °C for 5 h, the product was ultrasonic 10s and centrifuged to get the clear released supernatant for use.

The fabrication of the SERS capture probe was proceed as follows. 50 μL released supernatant was added into the solution mixed with DNA-MB complex suspension (50 μL), SERS signal probes (100 μL). The mixed solution was shaken at 37 °C for 1.5 h. Then, the excess SERS signal probes was removed by magnetic separation. After that, the SERS capture probe was washed with PBS for three times and dispersed into 50 μL 0.01M PBS for the following measurement.

2 μL MBs suspension was dropped onto the Au slices and air-dried at room temperature. The excitation laser, laser power and acquisition time for each spectrum were 633 nm, 5 mW and 10 s respectively. Three repeated experiments were carried out and the standard deviation was shown by error bars.

S2 The porosity of MSNs

The N_2 adsorption-desorption isotherm demonstrated that the obtained MSNs have a Burnauer-Emmett-Teller (BET)-surface area of 649 m^2 g^{-1} (Fig. S2A, ESI†) and a narrow Barrett-Joyner-Halenda (BJH) pore-size distribution (2.4 nm average pore diameter, Fig. S2B, ESI†).

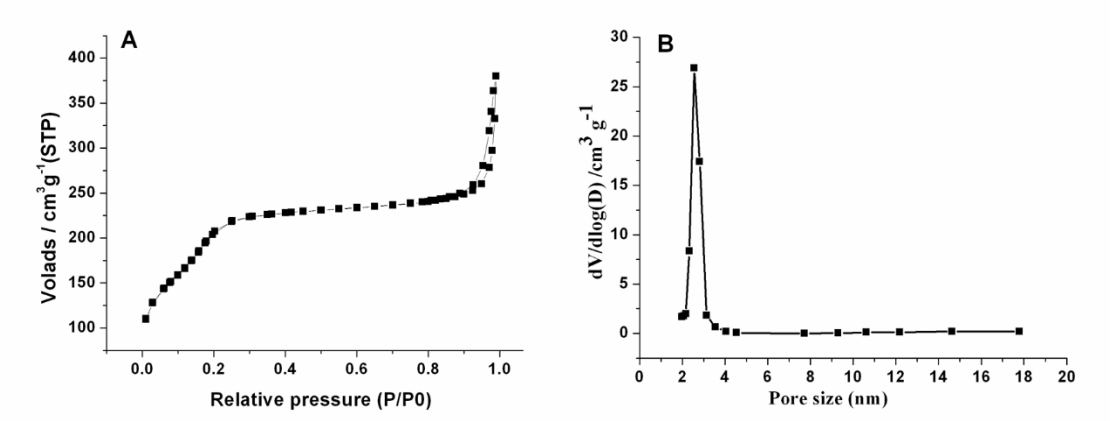


Fig. S2. (A) N_2 adsorption−desorption isotherm of MSNs；(B) Pore size distribution of MSNs.

S3 FTIR spectra of materials

The FTIR spectra of nanoparticles before and after modification were shown in Fig. S3.

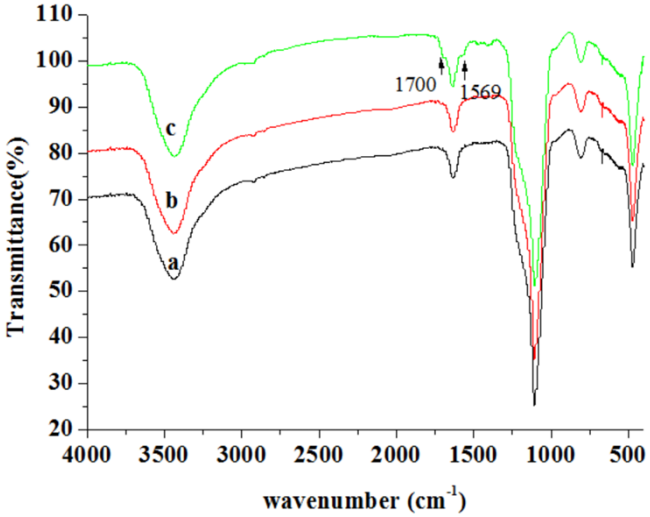
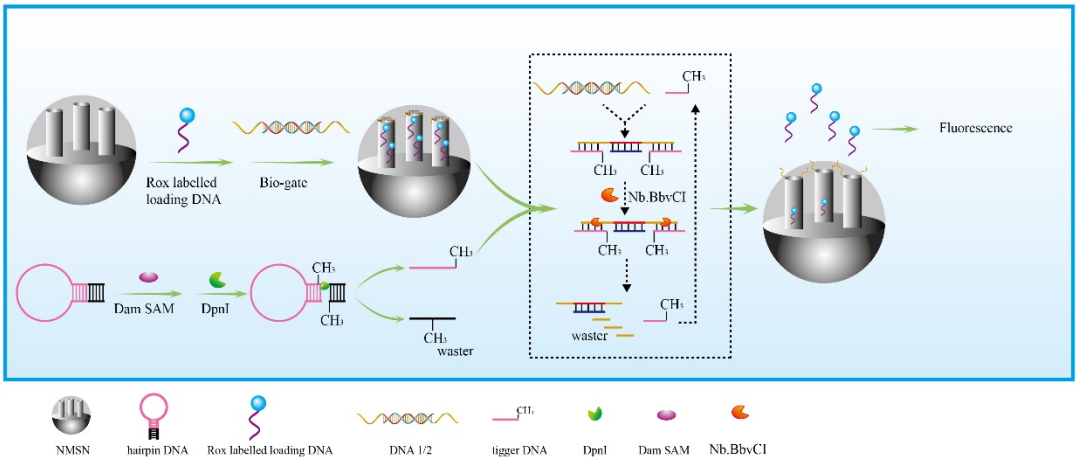


Fig. S3. FTIR spectra of materials before and after modification: (a) MSNs, (b) NMSNs, and (c) MSN-DNA-DNA1/2 nanoparticles.

S4 Optimization of the experimental conditions

The loading temperature and pH were optimized in order to maxmize the loading dosage. In this optimize step, Rox labelled loading DNA (RDNA) was used to replace loading DNA, as shown in Scheme S1.



Scheme S1. Schematic representation of the MSN probe for DNA MTases by fluorescence.

S4.1Optimization of the incubation pH and temperature

By measuring the fluorescence spectra of the RDNA in the stock solutions ($n_1=c_1*V_1$) and in the supernatant ($n_2=c_2*V_2$), the loading capacities of RDNA (C_{RDNA}) were calculated as follows: $C_{RDNA} = (n_1-n_2)/m_{NMSNs}$, where the units of n, c, V,

m, and C are mol, mol L^{-1}, L, g, and mmol g^{-1}. It was determined from the fluorescence specture showed in Fig. S3 and S4A that the maximum loading dosage is around 10^{-3} mmol g^{-1} at following three cases: (i) the stock solutions is 10^{-5} M, loading pH and temperature is 5.6 and 20 ºC, (ii) the stock solutions is 5×10^{-6} M, loading pH and temperature is 5.6 and 20 ºC, (iii) the stock solutions is 5×10^{-6} M, loading pH and temperature is 7.4 and 20 ºC.

MSN-Rox-DNA1/2 were incubated in 10U and 0U DNA MTases (0.1 M, pH 7.4 PBS) for 5 h. The RDNA released from the nanocarriers was collected by centrifugation at 10000 rpm, and measured by Fluorescence spectra. The incubation temperature influenced the release of loading DNA greatly. As the incubation temperature increased, the released loading DNA increased correspondingly (Fig. S4 A). As shown in Fig. S4 B (curve a), the RDNA release from the nanocarriers without DNA MTases is very few. The result also indicated that the loading pH also effect the release procedure. The release amount from the nanocarrier (loading pH 7.4) is two times as much as than the other one (loading pH 5.6). Hence, the loading and release temperature used in DNA Methyltransferase detection was 20 ºC and 37 ºC respectively. The incubation pH was 7.4 for both loading and release process.

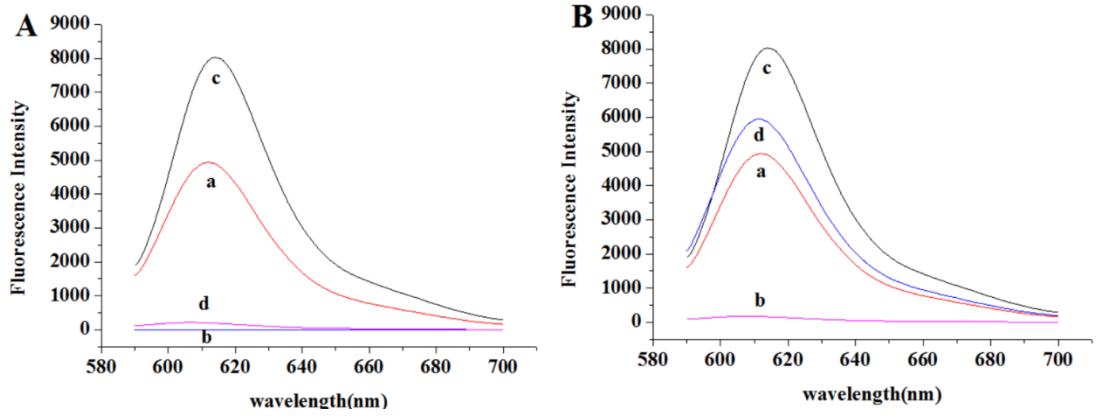


Fig. S4. (A) The Fluorescence spectra of (a) the stock solutions (5×10^{-6} M) and (b) supernatant, (c) the stock solutions (10^{-5} M) and (d) supernatant at pH5.6. (B) The Fluorescence spectra of (a) the stock solutions (5×10^{-6} M) and (b) supernatant, (c) the stock solutions (10^{-5} M) and (d) supernatant at pH7.4.

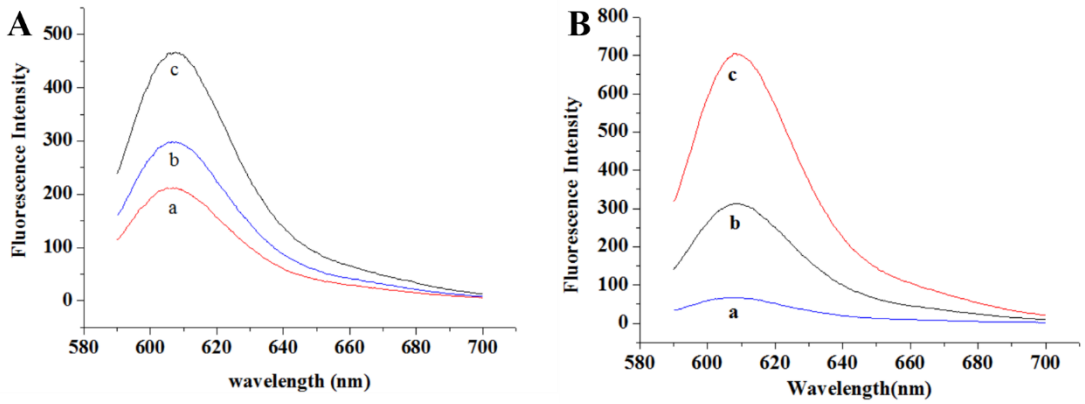


Fig. S5. (A) Fluorescence spectra of the supernatant at (a) 20 ºC, (b) 25 ºC, (c) 37ºC. (B) The Fluorescence spectra of the release RDNA by (a) 0U DNA MTases, (b)10U DNA MTases with MSN-Rox-DNA1/2 (loading pH 5.6) and (c) 10U DNA MTases with MSN-Rox-DNA1/2 (loading pH 7.4).

S4.2 Optimization of the incubation time

The incubation time was studied by performing the experiment at different time intervals to achieve the best sensing performance. As shown in Fig. S5, the Raman intensity rapidly increased within the first 2 hours. Then the growth was slowing down after that and nearly reached a plateau after 4 hours. Therefore, the reaction time was kept at 5 h in the experiment.

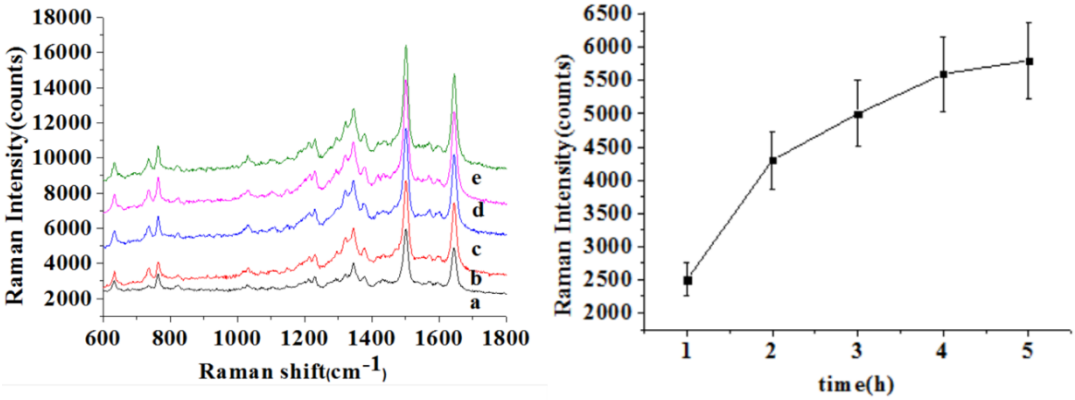


Fig. S6. Effect of the time of the reaction, DNA MTases concentration used here is 100U.

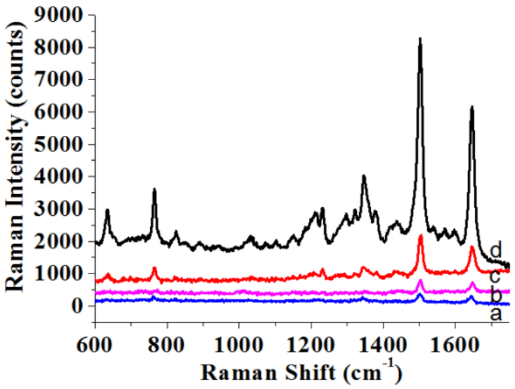


Fig. S7 SERS spectra obtained from MSN-DNA-DNA1/2 system: (a) in the absence of DNA Methyltransferase; (b) in the absence of Dpn I; (c) in the absence of Nb. BbvCI; (d) in the presence of DNA Methyltransferase (100U), Dpn I and Nb. BbvCI.

Table S2. Comparison of different methods for DNA MTases detection.

Method	Transducer	Detection limits (U)
Activity-Based DNA-Gold Nanoparticle Probe	Colorimetry	0.7 U/mL[7]
Graphene Oxide Combining with Restriction Endonuclease	electrochemical method	(0.05 ± 0.02) U/ mL[8]
methylation-triggered DNAzyme-based DNA machine	Colorimetry	0.25 U/ mL [9]
DNAzyme-Based Amplified Biosensing Platforms	Fluorescence	0.2 U/ mL [10]
Methylation-blocked enzymatic recycling amplification	Fluorescence	0.05U/ mL [11]
A Raman strategy with the combination of HCR	SERS	0.067 U/ mL [12]
This work	MSN-SERS	0.02 U/mL

S5 References

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