Electronic Supplementary Information (ESI)

for

Nanoscale DNA-Au Dendrimer as a Signal Amplifier for Universal Design of Functional DNA-based SERS Biosensors

Yun-Hong Sun,[a] Rong-Mei Kong,[a] Dan-Qing Lu,[a] Xiao-Bing Zhang,*[a] Hong-Min Meng,[a] Weihong Tan,*[a,b] Guo-Li Shen,[a] and Ru-Qin Yu[a]

[a] *State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha 410082, China. E-mail: xbzhang@hnu.cn*

[b] *Department of Chemistry and Shands Cancer Center, UF Genetics Institute and McKnight Brain Institute, University of Florida, Gainesville, Florida 32611, USA. E-mail: tan@chem.ufl.edu*

Table of Contents

1. Experimental Details

Materials and Apparatus. All oligonucleotide samples were purchased from Takara Biotechnology Co., Ltd. (Dalian, China). Adenosine, cytidine, uridine, guanosine, 6-mercaptohexanol (MCH) and tri(2-carboxyethyl)phosphine hydrochloride (TCEP) were all purchased from Sigma-Aldrich. Lead acetate, $HAuCl_4 \cdot 3H_2O$, NH_2OH, trisodium citrate, and other reagents were of analytical reagent grade, were purchased from China National Medicines Co. Ltd., and were used without further purification. Solutions of Pb^{2+}, Zn^{2+}, Cd^{2+}, Co^{2+}, Ni^{2+}, $Sr^{2+}$and Hg^{2+} were prepared from their nitrate salts; solutions of Cu^{2+}, Fe^{2+}, Mn^{2+} and Ca^{2+} were prepared from their chloride salts. All solutions were prepared in Milli-Q water (resistance >18 MΩ·cm) from a Millipore system. UV-Vis absorption spectra were recorded with a Shimadzu MultiSpec-1501 spectrophotometer. Fluorescence measurements were carried out on a Hitachi F-7000 fluorescence spectrometer with excitation slit set at 5.0 nm and emission at 5.0 nm. The pH measurements were carried out on a Mettler-Toledo Delta 320 pH meter.

For Raman measurements, a confocal microprobe Raman instrument (RamLab-010, Jobin Yvon Horiba, France) was used. A 632.8 nm He-Ne laser excitation (1.2 mW) and a 50 × long working-distance objective (8 mm) were used in this work. The width of the slit and the size of the pinhole were set as 100 and 1000 μm, respectively. Raman mapping was carried out with an area of 2 μm × 2 μm. The collection time for each Raman spectrum is 10 s. Atomic Force Microscopy (AFM) images were performed on a multimode AFM34 having a piezoscanner with a maximum scan range of 100 μm × 100 μm × 5 μm.

The sequences of oligonucleotides used in this work are given in Table S1. GR-5 E-S contains both enzyme and substrate sequences of GR-5 DNAzyme linked by 10 T bases. It is also modified with a SH group on its 3' terminal. Reporter DNA **1** is partially complementary (12 base pairs) with the GR-5 E-S. The complement of Reporter DNA **1** is denoted as Reporter DNA **2**. Besides the 3' SH group, reporter

DNA **1** and **2** contain a 5′ Rox (X-rhodamine) sulfhydryl group. Rox with the absorption and fluorescence peak at 585 nm and 601 nm, respectively, was selected as the Raman probe signal. GR-5 E-S and Reporter DNA **1** and **2** constitute the SERS sensing system for Pb^{2+}. The aptamer-based SERS detection system for adenosine is comprised of four components, including AD-aptamer, Capture probe, Reporter DNA **1**, and Reporter DNA **2**. Capture probe contains a 3′ SH group and is partially complementary with both the AD-aptamer (with 12 base pairs) and the Reporter DNA **2** (with 11 base pairs).

Table S1. Sequences of oligonucleotides used in this work[a]

Oligonucleotide	Sequences (from 5′ to 3′)
GR-5 E-S	CATATCTCTGAAGTAGCGCCGCCGTATAGTGAGTTTTTTTTTTC TCACTATrAGGAAGAGATATGTT-$(CH_2)_3$-SH
AD-aptamer	AGAGA <u>ACCTGGGGGAGTATTGCGGAGGAAGGT</u>
Capture probe	CCCAGGT TCTCT TCCACT-($CH_2)_3$-SH
Reporter DNA **1**	Rox-CATATCTCT TCCACT-$(CH_2)_3$-SH
Reporter DNA **2**	Rox-AGTGGAAGAGATATG-$(CH_2)_3$-SH

[a] The rA denotes adenosine ribonucleotide at that position while all others are deoxyribonucleotides. The underlined sequences in AD-aptamer represent the original sequence of anti-adenosine aptamer.

Synthesis of AuNPs. In general, AuNPs with smaller size show weaker SERS activity than AgNPs or bigger AuNPs.[S1] AuNPs of ~13 nm in diameter were therefore chosen as building blocks for constructing the Raman sensing platform, since they were expected to afford a low background SERS signal induced by the nonspecific adsorption of NPs. They were prepared via the reduction of $HAuCl_4$ by sodium citrate.[S2] Glassware used in the preparation was soaked in aqua regia and rinsed thoroughly with Millipore water. In a 250-mL two-neck flask, 2 ml of 50 mM $HAuCl_4$ solution was added into 98 mL of Millipore water and heated to reflux under vigorous stirring; 10 mL of 38.8 mM sodium citrate solution was then added into the boiling solution. Within several minutes, the color of the reaction mixture changed from pale yellow to wine-red. After refluxing for another 20 min under stirring to

allow complete reduction, the solution was cooled slowly to room temperature. Then it was filtered through a 320-nm membrane filter and stored in a refrigerator at 4 °C before being used. The concentration of AuNPs was estimated by UV/vis spectroscopy to be about 13 nm, based on an extinction coefficient of 2.7×10^8 $M^{-1}cm^{-1}$ at λ =520 nm for 13-nm particles.[S3]

Functionalization of AuNPs with reporter DNA.[S4] The reporter DNA (1 or 2) with a 3' SH group was first activated by incubating with TCEP. Typically, 50 μL of 30 μM DNA, 5 μL of 10 mM TCEP and 50 μL of 100 mM acetate buffer (pH 5.2) were mixed in a microcentrifuge tube and incubated at room temperature for 1 hour. The mixture was then directly added into 500 μL of the above prepared AuNPs in a 1.5 mL microcentrifuge tube. After incubation in the dark for 16 hours at room temperature, 32 μL of buffer containing 2 M of NaCl and 100 mM of Tris acetate (pH 8.2) was added dropwise to the nanoparticle solution with gentle hand shaking. After incubation in a dark place for another day, the so-resultant solution was centrifuged at 12000 rpm on a benchtop centrifuge for 20 minutes. The supernatant was removed and nanoparticles were re-dispersed in buffer containing 100 mM NaCl and 25 mM Tris acetate (pH 8.2). This centrifugation process was then repeated for 2 times to remove most of the free DNA in solution. The final solution was re-dispersed in the buffer solution (pH 8.2, 25 mM Tris acetate, 100 mM NaCl) and stored at 4 °C.

Sensing Interface Preparation. A gold electrode (99.99% polycrystalline gold, ~2 mm diameter) was chosen as the Raman substrate to fabricate a SERS sensing interface. It was polished on a microcloth sequentially with 0.3 and 0.05 μm alumina slurry and cleaned in an ultrasonic bath successively with double distilled water, absolute alcohol, and Milli-Q water for 5 min each. Subsequently, the gold electrode was immersed in piranha solution (H_2SO_4/H_2O_2, 7:3 by volume) for 20 min, rinsed with Milli-Q water and dried in a nitrogen atmosphere. To construct a Pb^{2+} sensing interface, the resulting gold electrode was incubated in a 10 mM Tris-HCl buffer solution (pH = 7.2, 5 mM $MgCl_2$ and 100 mM NaCl) containing 0.1 μM thiolated DNAzyme-substrate for 16 h, and then in same buffer solution containing 0.1 μM 6-mercaptohexanol (MCH) for 20 min at 37 °C. The electrode surface was then rinsed with the blank Tris-HCl buffer at 50 °C for 20 min to remove the nonspecific adsorbed thiolated

oligonuleotide and MCH, rinsed with Milli-Q water and dried under a stream of nitrogen. MCH was introduced to block the nonspecific absorption sites on the substrate surface, and provide an optimal oligonucleotide orientation, which is favorable for the binding of DNAzyme with its cofactor Pb^{2+}, the sequential release of the enzymatic cleavage product, and the subsequent hybridization of the remained oligonuleotide moiety on the electrode surface with the reporter DNA **1** strand.

A similar procedure was conducted to fabricate the adenosine sensing interface, except for the use of a short thiolated capture probe hybridized with partial sequences of an anti-adenosine aptamer to replace the thiolated DNAzyme-substrate to be immobilized on the SERS substrate surface.

Target detection. For sensing assay of Pb^{2+}, the DNAzyme-substrate strand on the electrode surface was annealed in a 10 mM Tris-HCl buffer (pH 7.2, containing 100 mM NaCl and 5 mM $MgCl_2$) solution by warming the solution to 95°C for 5 min and subsequently cooling in ice water before use. To detect Pb^{2+} or other metal ions, the electrode was then immersed in 200 μL of buffer solution containing Pb^{2+} of specified concentration or other metal ions for 20 min at 37 °C, followed by washing for 1 min with the blank buffer solution. The electrode was consequently incubated in reporter DNA **1**- and reporter DNA **2**-functionalized AuNPs solution for 30 min at 37 °C and then rinsed with buffer for 2 min. The above procedure was repeated 4 times, and the obtained electrode was dried at nitrogen atmosphere for SERS measurement.

To detect adenosine, the prepared adenosine sensing electrode was immersed in 200 μL of 10 mM PBS buffer (pH 7.4) solution containing 300 mM NaCl and adenosine of specified concentration for 20 min at 37 °C followed by washing for 1 min with the blank buffer solution. Then, the electrode was sequentially interacted with AuNPs-functionalized reporter DNA **2** and reporter DNA **1** for 30 min at 37 °C, and then rinsed with buffer for 2 min. After repeating this procedure 4 times, the modified electrode was dried at nitrogen atmosphere for SERS measurement.

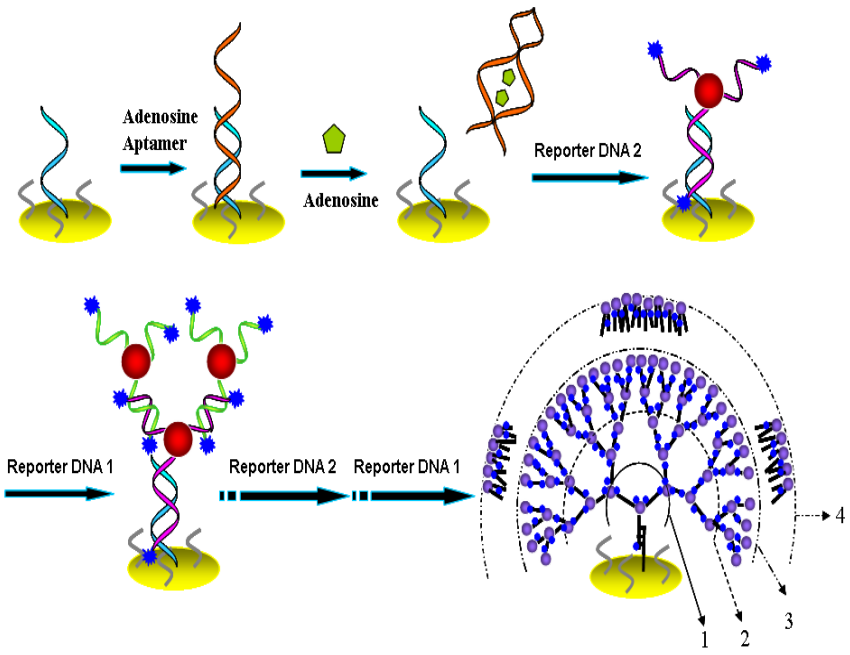
2. Effect of layers

To investigate the amplified effect of the DNA-Au dendrimer, the concentration of Pb^{2+} was fixed at 200 nM, and the SERS signal was then recorded with the introduction of different layers of reporter DNA-functionalized AuNPs. In the absence of reporter DNA-functionalized AuNPs, no obvious Raman signal was observed (the 0 curve in Fig. 1a) as a result of the small Raman scattering signal from DNA molecules themselves together with the low SERS enhancement induced by the smooth Au electrode surface. The introduction of the first reporter DNA **1**-functionalized AuNPs layer to hybridize with the remaining oligonuleotide moiety on the electrode surface triggered a weak but distinct SERS signal (the 0.5 curve in Fig. 1a) with peaks centered at ca. 1345, 1499, and 1642 cm^{-1}. These SERS peaks are very similar to those obtained for Rhodamine dyes adsorbed on noble metallic surfaces,[S5-S7] and, as such, they could be assigned to the aromatic C–C stretching vibrations of Rox (X-rhodamine) (see Fig. S1).[S8-S10] The strongest Raman band at 1499 cm^{-1} was selected as the characteristic peak for subsequent quantitative investigation. With the further introduction of the reporter DNA **2**-functionalized AuNPs to hybridize with the free reporter DNA **1** on the AuNPs to form the first layer of nanoscale dendrimer, the Raman signal enhanced remarkably by the sharp decrease of the distance between the AuNPs and Rox tag, and that between two conjugated AuNPs, which switched on the hot spot-induced EM coupling effect (the 1 curve in Fig. 1a). As illustrated in Scheme 1, the steric effect between AuNPs resulted in a phenomenon in which more than one reporter DNA **2**-functionalized AuNP could hybridize with one reporter DNA **1**-functionalized AuNP to form a dendrimer structure, which would further amplify the Raman signal of Rox. With the cooperation of the two effects, a 400% Raman signal enhancement at 1499 cm^{-1} in comparison with that of "0.5" case was observed upon the introduction of reporter DNA **2**-functionalized AuNPs to form the "1" layer of DNA-Au dendrimer (Fig. 1e). The repeated introduction of reporter DNA **1-** and **2**-functionalized AuNPs could continuously amplify the Raman signal. The largest signal enhancement was observed with the formation of "4" layers of DNA-Au dendrimer,

which showed a 1750% Raman signal enhancement at 1499 cm^{-1} in comparison with that of "0.5" case (Fig. 1e).

The formation of such nanoscale dendrimer on the "smooth" Au surface is further confirmed by AFM images, as shown in Fig. S2a–c. One can clearly observe the appearance and growth process of nano-hunches on the initial flat "smooth" Au surface (Fig. S2a). As the DNA-Au dendrimer on the gold surface change from one to four layers, the size of the nano-hunches is obviously increased (Fig. S2b, S2c). The uneven size distribution of these nano-hunches might be caused by the random distribution of the capture probes on the "smooth" Au surface, which is definitely not a perfectly uniform substrate.

3. Supplementary Figures



Scheme S1 Schematic of the amplified sensing strategy of the DNA-Au dendrimer-based, structure-switching SERS aptamer biosensor for adenosine.

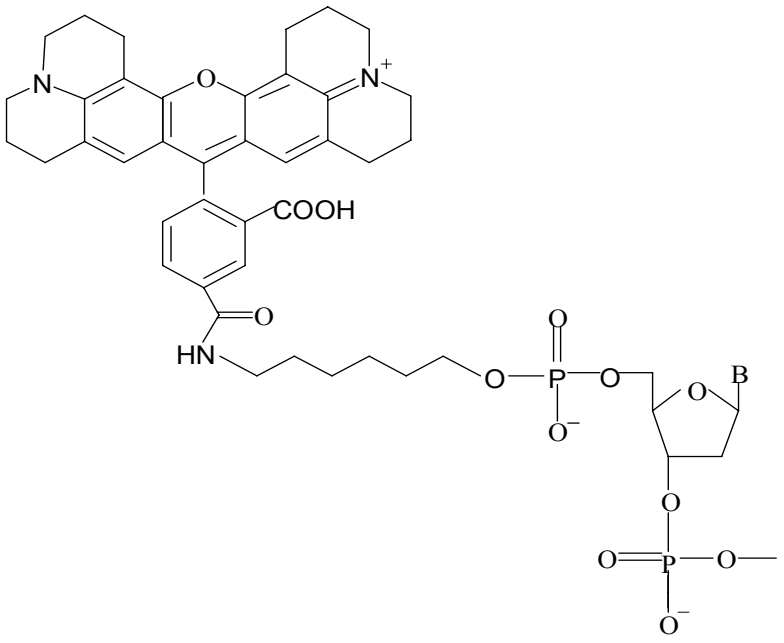


Fig. S1 Structure of Rox (X-rhodamine)

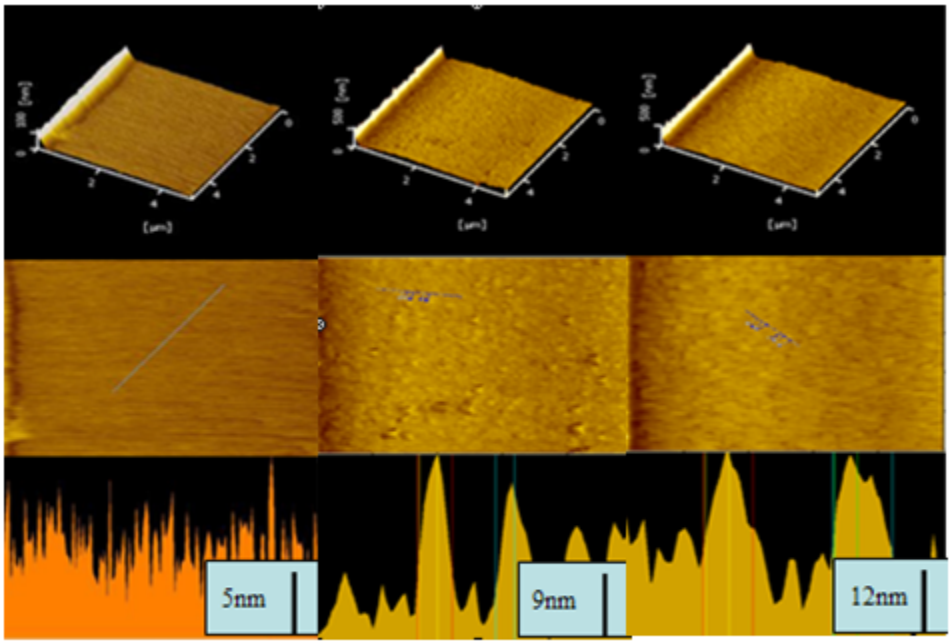


Fig. S2 3D images and height of AFM images of: (a) the blank gold surface; (b) after being modified with one (reporter DNA **1**+**2**) layer; (c) after being modified with four layers. The Pb^{2+} concentration was fixed at 200 nM.

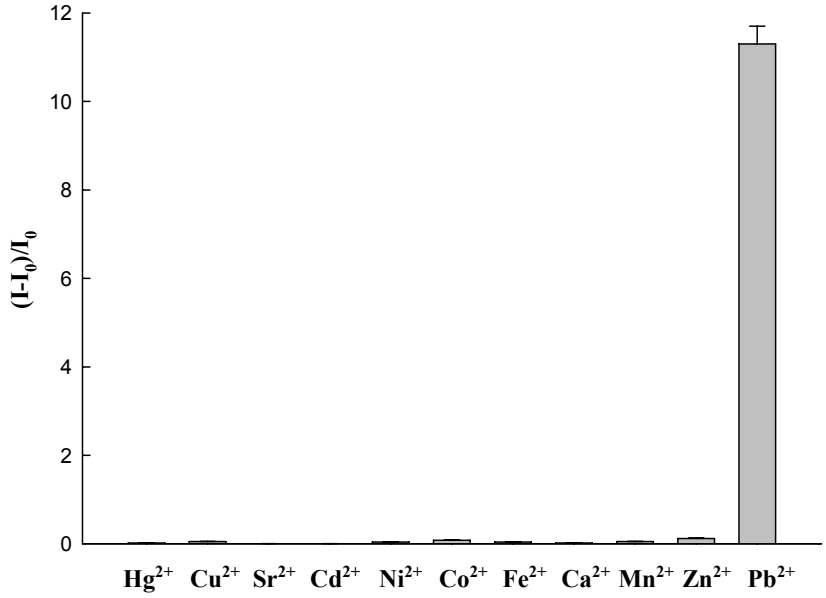


Fig. S3 Selectivity of DNA-Au dendrimer-based SERS DNAzyme sensor for Pb^{2+} over other competing divalent metal ions. The ratios of SERS intensity enhancement at 1499 cm^{-1} of the sensing system induced by different metal ions (200 nM) to that of the blank are shown. The buffer solution contained 10 mM Tris-HCl (pH 7.2), 100 mM NaCl and 5 mM $MgCl_2$.

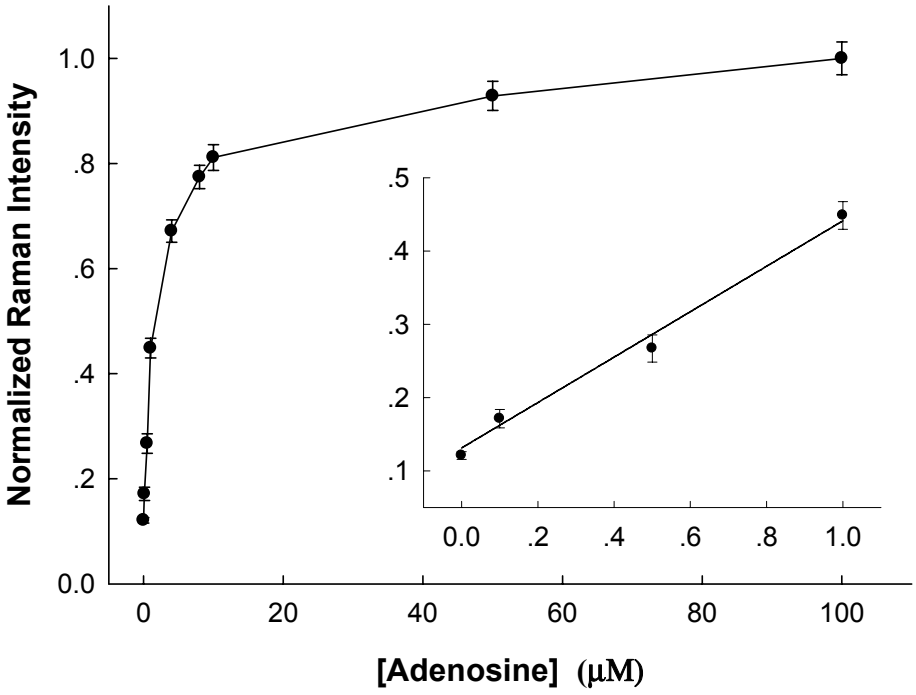


Fig. S4. Calibration curve of the structure-switching aptamer SERS biosensor for adenosine. The curve was plotted with the normalized Raman intensity at 1499 cm^{-1} vs. adenosine concentration. The average intensity at the adenosine concentration of 100 μM was set as "1". Inset shows the linear responses at low adenosine concentrations.

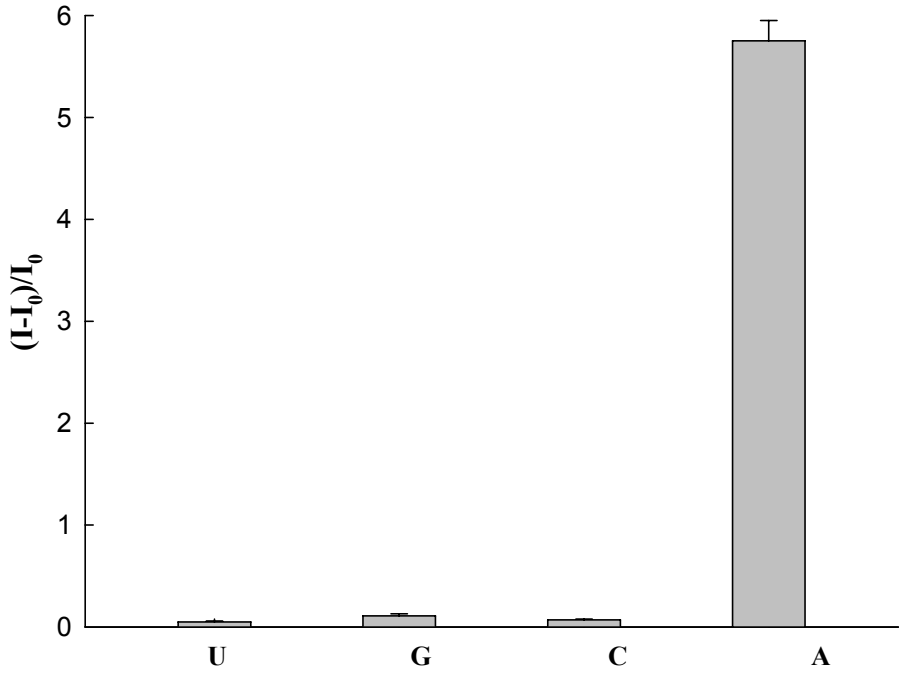


Fig. S5 Selectivity of DNA-Au dendrimer-based SERS aptamer sensor for adenosine over other competing species. The ratios of SERS intensity enhancement at 1499 cm^{-1} of the sensing system induced by different species (10 μM) to that of the blank are shown. The buffer solution contained 10 mM PBS (pH 7.4) and 300 mM NaCl.

4. References

S1 J. Hu, P. C. Zheng, J. H. Jiang, G. L. Shen, R. Q.Yu, and G. K. Liu, *Analyst,* 2010, **135**, 1084.

S2 K. C. Grabar, R. G. Freeman, M. B. Hommer, and M. J. Natan, *Anal. Chem.,* 1995, **67***,* 735.

S3 L. M. Demers, C. A. Mirkin, R. A. Mucic, R. L. Reynolds, R. Letsinger, R. Lghanian, and G. Viswanadham, *Anal. Chem.,* 2000, **72***,* 5535.

S4 J. Liu, and Y. Lu, *Nature Protoc.,* 2006, **1**, 246.

S5 Merlen, A.; Lagugne-Labarthet, F.; Harte, E. *J. Phys. Chem. C,* 2010**, 114***,* 12878.

S6 Y. Liu, L. Liu, and R,Guo. *Langmuir,* 2010, **26**, 13479.

S7 A. M. Michaels, M. Nirmal, L. E. Brus, *J. Am. Chem. Soc.,* 1999, **121**, 9932.

S8 P. Hildebrandt, and M.Stockburger, *J. Phys. Chem.* 1984, **88**, 5935.

S9 M. Culha, D. Stokes, L. R. Allain, and T. Vo-Dinh, *Anal. Chem.* 2003, **75,** 6196.

S10 Y. Lu, G. L. Liu, and L. P. Lee, *Nano Lett.* 2005, *5*, 5.