Electronic Supplementary Material (ESI) for Chemical Communications

Electronic Supplementary Information

Surface-enhanced Raman Scattering Assay Combined with Autonomous DNA Machine for Detection of Specific DNA and Cancer Cells

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Experimental

Reagents. All DNA oligonucleotides (Table S1) were purchased from SBS Genetech. Co. Ltd. (China). The RepliPHI Phi29 DNA polymerization, deoxyribonucleoside T4 DNA ligase and 10× T4L DNA ligase buffer, Nb. BbvCI (10000U/mL) and deoxyribonucleoside 5′-triphosphates (dNTPs) mixture were abtained TaKaRa Bio Inc. Mercaptoacetic acid, imidazole, and 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) were also purchased from Sigma-Aldrich. The carboxyl-modified MBs (size: 1.0~2.0 μm) were purchased from Tianjin Baseline ChromTech Research Centre (China). Solutions were prepared with Milli-Q deionized water. The pH of all buffers was adjusted with either NaOH or HCl solution. The Au slides were purchased from Oy BioNavis Ltd. All other reagents were of analytical grade.

Table 1 DNA sequence used in this work

Oligonucleotides name	Sequences	Discription
Hairpin DNA	5-NH_2-T_{15}-GTG TTC AGT GTG TGG TGC TGA GGA AGT AGA CCC TGA ACA G-3	Hairpin DNA immobilized on MB-A in scheme-1A
Ramos aptamer (template)	5-AAC ACC GTG GAG GAT AGT TCG GTG GCT GTT CAG GGT CTC CTC CCG GTG -NH_2-3	Ramos aptamer (template) for the strand displacement reactions immobilized on MB-C in scheme 1A
Target DNA 1	5-ATC CTC CAC GGT GTT CCT CAG CAC CAC G-3	complementary to Ramos aptamer (template) and underline means a Nt. BbvCI recognition region.
Padlock DNA	5-PO_4-ACT GAA CAC CCC AAC CCG CCC TAC CCA AAA CCC AAC CCG CCC TAC CCG CAC CAC AC-3	padlock DNA and underline means complementary to DNA 3 (the block region of hairpin DNA)
Signal DNA	5-Rox-TTT TTT CCT AGC GAC-SH-3	The signal probes immobized on the Au nanoparticle
Capture probe	5-CCC AAC CCG CCC TAC CCT TTT TT -SH-3	The capture probe immobized on the Au nanoparticle
Probe-A	5-TTT TTT TTT TTT TTT TTT TTT TTT TTT T-3	Non complementary DNA
Probe-B	5-ATC CTC CAC GGT GTT CCT CAC CAC CAC G-3	One base mismatched DNA-1(the base mismatched in the Nt. BbvCI recognition region.
Probe-C	5-ATC CTC CAC GGT GAT CCT CAG CAC CAC G-3	One base mismatched DNA-2

Table S1 DNA Sequence Used in This Work

Apparatus. Raman measurements were conducted in 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 spectra were calibrated with the WiRE Raman Software Version 2.0 from Renisaw Ltd. Transmission electron microscopy (TEM) images were recorded on a JEM 1200EX trans-mission electron microscope (JEOL, Japan). UV/Vis absorption spectra were obtained with a Cary 50 Series Spectrophotometer (Varian, Australia).

Preparation of Au NPs. Au NPs were prepared according to the method reported[1] previously with a slight modification. 50 mL of 0.01% $HAuCl_4$ solution was heated and stirred to reflux in a three neck round bottomed flask. After boiling of the solution, 1.0 mL of 1% trisodium citrate was immediately added to the $HAuCl_4$ solution. The temperature of boiling point was maintained at reflux for 20 min, during which time deep red coloration formed. After that the solution was allowed to cool to room temperature. The final gold nanoparticles prepared by this method had an average diameter of approximately 20 nm by TEM (figure S3). The prepared gold nanoparticles were stored in brown glass bottles at 4 □.

Preparation of Bio Barcoded Au NPs. The process of bio bar coded Au NPs according a literature[2] was enforced as follows: The 50 μL of mixtures of signal DNA and 10 μL of capture DNA (shown in table 1) with different molar ratios (30:1, 40:1, 50:1, 60:1, 70:1) were activated with 2.0 μL of 10 mM TCEP for 1 h, and then added to 1 mL of freshly prepared gold nanoparticles with gentle shaking overnight. Over this course, the DNA-AuNP conjugates were aged in salts (0.1 M NaCl, 10 mM acetate buffer) for another 24 h. The additional reagents were removed by centrifuging at 10000 rpm for 30 min. The red precipitate was washed and centrifuged repeatly for three times. The resulting nanoparticles were dispersed into a buffer solution (pH 8.2)

and stored at 4 □. The clear supernatant was carefully removed, and the precipitated probes were redispersed in 10 mL of the 0.01 mol L-1 phosphate buffer solution (PBS, pH 7.40, 0.10 mol L-1 NaCl + 0.01 mol L-1 KH_2PO_4/ K_2HPO_4) and stored at 4 □.

Ramos Cell Culture. Ramos cells (CRL-1596, B-cell, human Burkitt's lymphoma) were procured from the Chinese Academy of Medical Sciences. The cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 100 IU mL^{-1} penicillin Streptomycin. The cell density was determined by using a hemocytometer, and this was performed prior to any experiments. Approximately one million cells dispersed in RPMI 1640 cell media buffer were centrifuged at 3500 rpm for 5 min and were then redispersed in PBS buffer (1 mL, 0.1 M, pH 7.4). During all experiments, the cells were kept in an ice bath at 4 ℃.

Immobilization of Probes onto MBs. First, 10 μL of carboxyl-modified MBs suspension was previously washed with 400 μL of 0.1 M imidazol-HCl buffer (pH 6.8) three times, followed by activation in imidazol-HCl buffer (200 μL, 0.1 M, pH 6.8) containing EDC (0.1 M) with gentle shaking for 30 min. And then capture DNA, template DNA or hairpin probe DNA was separately added into the activated MBs (MB-A, MB-B, MB-C), and the resultant mixture was incubated for 12 h at 37℃ with gentle shaking. Finally, the additional capture probes were removed by magnetic separation. The resulting capture probes conjugated MBs were washed with 200 μL of 0.01 M PBS buffer (pH 7.0) three times and resuspended in 200 μL of PBS buffer and kept at 4 ℃.

Assay Process. 50 μL of capture probe-modified MBs suspension was incubated for 60 min with 30 μL of target DNA 1 (aptamer DNA) in 37 °C water bath, magnetically separated, and mixed with template probe-modified MBs and hairpin probe-modified MBs in a tube. 1 μL Exonuclease-free Klenow (Klenow fragment, exo-) polymerase, the nucleotide

mixture(deoxynucleotide triphosphate (dNTPs) act as fuel and 0.5 μL N.BbvCI nicking endonuclease, which nick the single strand at the marked position, were added. Then Klenow polymerase, Ramos cells, dNTPs and N.BbvC IA nicking endonuclease which nick the single strand at the marked position and Ramos cells were added. The resultant mixture was incubated for 2 h at 37 °C. After that the mixture was washed three times with PBS buffer and the single strand on MBs were left by magnetic separation.

RCA Reaction. Next, 10 μL of padlock probe solution was introduced to DNA-2 on MBs, heated up to about 95 °C and then cooled down to room temperature. Then, T4 DNA ligase, 5 μL 10 mM dNTPs and 1 μL 10 U/μL Phi29 DNA polymerase followed was added. The ligation was allowed to proceed in a 22 °C water bath for 0.5 h. Subsequently, the RCA reaction was carried out with the addition of 5 μL 10 mM dNTPs, 1 μL 10 U/μL Phi29 DNA polymerase, 50 μL bio barcodes and the resulting MBs followed by incubation at 37 °C for 1 h..

Measurement of Raman Spectrum. Finally, the excess bio barcodes were removed by magnetic separation. The resulting MBs were washed with 200 μL of 0.01 M PBS buffer three times and dispersed in 50 μL of 0.01 M PBS buffer. After casting 3 μL of each solution onto the Au slides, air-dried at room temperature ahead of SERS analysis. SERS spectra were measured by Renisaw Raman spectrometer with a 633 nm laser. The laser power was 5 mW, and the resolution of SERS spectra over cm^{-1}. The collection time for each spectrum was 10 s. Three spectra from different sites was collected from each sample and averaged to represent the SERS results, and three repeated experiments were performed. Error bars showed the standard deviation of three experiments.

Results and discussion

UV-visible spectra of the Rox-DNA conjugates. The UV-visible spectra of capture DNA probe, Rox-DNA (signal DNA), Au NPs and Rox-DNA modified Au NPs conjugate were recorded by the spectrophotometer as shown in figure S1. Curve a, b, d exhibited respectively the characteristic absorbance of DNA at about 260nm (Curve a), Au NPs (curve c) at about 520 nm and Rox-DNA (curve b) at 260 nm and two characteristic absorbance at 500-600 nm consistent with the literature.[3] The appearance of characteristic absorbance of Au NPs at about 520nm as displayed in (Curve d) indicated that Rox-DNA had successfully conjugated with Au NPs on the 3'- terminus.

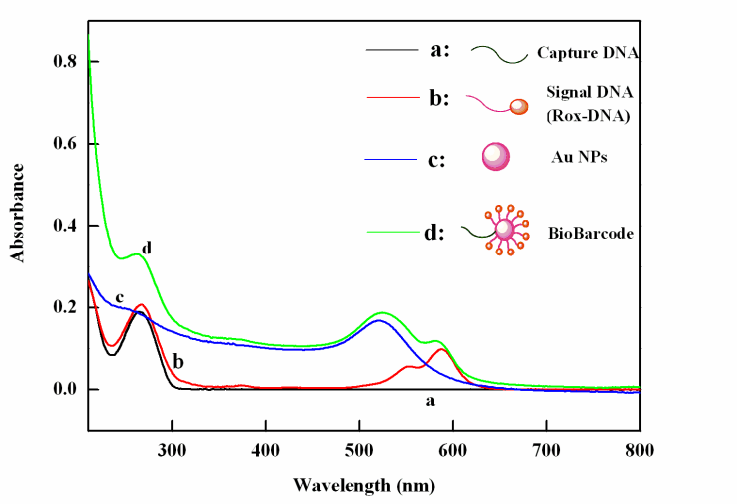

Figure S1. UV spectra of DNA (a), Rox-DNA (b), Au colloid (c), and bio barcode DNA (d).

The feasibility of the DNA machine amplification. The comparison of the Raman intensity of the present method and the RCA method were investigated in presence of 10^{-14} M target DNA. Due to the cycle 1 is triggered once target DNA binding to the hairpin DNA, the cycle 1 can not skipped in the RCA method. As shown in figure S2, the SERS intensity increased with the increasing of prolong time of RCA, implying the longer the RCA, the longer single stranded DNA should be and more SERS probes binding therefore increasing the SERS signal. However, the signal intensity of the present method accomplishing two cycles and RCA was higher than that of

control method which skipped cycle 2. The target DNA 1 was released through the polymerization of cycle 2, which stimulate the generation of enhanced Raman signal. This indicates the feasibility of double cycles amplification mechanism of the strategy.

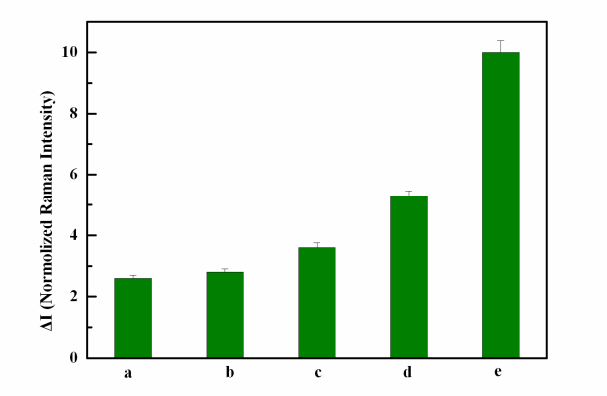

Figure S2. SERS spectra obtained from DNA machine with completing cycle 1 and RCA reaction with the prolong time: 2 min (a), 5 min (b), 10 min (c), 20 min (d), with completing cycle 1 and cycle 2 and RCA reaction (e), target DNA 1 concentration, 10^{-14} M.

Optimization of the reaction conditions. In order to establish optimum conditions for the operation of DNA machine, some important parameters were investigated systematically. The DNA machine system involves several kinds of reactions, such as competition for DNA binding enzymatic reaction, dissociation of dsDNA hybrids in solution, the polymerization and nicking process.

Optimization of the temperature and pH of the cycle reaction. Taking account of the amplification efficiency of DNA machine, the effect of pH of the PBS buffer solution for cycle reaction was investigated in the pH range from 6.5 to 8.0 as shown in figure S3a. ΔI reached the maximum at pH 7.4. Thus, the pH 7.4 was used as an optimal condition.

As is well known, high temperature is disadvantageous to DNA hybridization, but is advantageous to dissociation of dsDNA hybrids. The polymerization and nicking enzymatic reactions are usually operated at 37 ^{o}C by virtue of the best bioactivity of enzymes. In order to balance these

coexistent reactions, the influence of reaction temperature was investigated. Figure S3b showed the changes of SERS intensity generated by the DNA machine system at different reaction temperature, upon analyzing target DNA 1 at a concentration corresponding to 10^{-14} M. The 37 ^{o}C was chosed as an optimal condition as shown in figure S3b.

Optimization of the time of the DNA machine operation and RCA reaction. In DNA machine system design, two cycle modes coexist in this system to amplify signals, so the consecutive reactions will not end until the substrates (fuels) are used up. A large number of DNA 3 is released as a RCA primer in the process of the cycle reaction, and the RCA is initiated to generate many repeated units sequences for binding the SERS active bio-barcodes. The SERS intensity was dependent on the amount of the SERS active bio-barcodes bound to the RCA product, the greater the number of DNA 3 produced by DNA machine cycle reaction, the greater the amount of the bound bio-barcodes. This is a time-dependent system, and the reaction time is an important influencing factor. Thus, in order to obtain higher sensitivity, the time of the DNA machine operation and RCA reaction were investigated, figure S3c showed the changes of the SERS intensity generated by operating the machine system at different time intervals. The result showed that the SERS intensity increased with the prolonging of reaction time, and apparent increases of Raman signals appeared within 2 h. Therefore, the reaction time of the system was chose to be 2 h. In order to obtain higher sensitivity, the RCA duration time was investigated. The results are shown in figure S3d. Under a higher concentration of phi29 polymerase and substrates, the Raman intensity increased rapidly with the RCA reaction time from 10 min to 1 h but tended to saturate over 1 h. Therefore, 1 h was selected as the optimum time for the RCA reaction. The results

showed that the reaction times of 2 h and 1 h were suitable for the DNA machine reaction and RCA reaction, respectively, and employed in the subsequent work.

Optimization of the concentration of the hairpin DNA immobilized on the MB. In DNA machine design, the hairpin DNA consists of two regions, once hybridized with target DNA 1. The duplex regions between 2 and 3 are thermally unstable, and they dissociate to form two single DNA chain 2, 3, DNA 2, which lead cycle 2; DNA 3, as the RCA primer, initiated the RCA reaction, thus the immobilization of the hairpin probes and an SERS signal-producing compound on the surface of a transducer plays an important role because the working potential and sensitivity are dependent upon these factors. Figure S3e shows the variance of SERS intensity with the concentration of hairpin probes. It can be seen that the SERS intensity increases with the increase of hairpin DNA concentration from 1.0×10^{-9} M to 1.0×10^{-5} M, and reaches a maximum at 1.0×10^{-7} M, upon analyzing target DNA 1 at a concentration corresponding to 1×10^{-14} M. These results demonstrated that low concentration hairpin probes might not hybridize with sufficient target DNA 1, but high-concentration hairpin probes might increase the steric hindrance of the microenvironment, adversely preventing their hybridization with target DNA 1[4]. Therefore, the concentration hairpin probes of 1.0×10^{-7} M was employed in the following work.

Optimization of the proportion of the signal probes and the capture probe immobized on the Au NPs. The Raman intensity is also influenced by the proportion of the signal DNA probes and the capture probe immobized on the Au NPs in the preparation of bio barcode probe. To improve the sensitivity of SERS quantification of DNA, the proportion of the capture probe and the signal DNA probes was optimized. Figure S3f shows the variance of Raman intensity with the proportion of the signal DNA probes and the capture probe. It was obviously that Raman intensity

increased upon raising the proportion from 30:1 to 50:1, and then it started to level off, attributed to the steric and electrostatic hinderance arising from the more tightly packed probe, which were necessary for highly hybridization efficiency. Thus the ratio of 50:1 was selected for the subsequent assays.

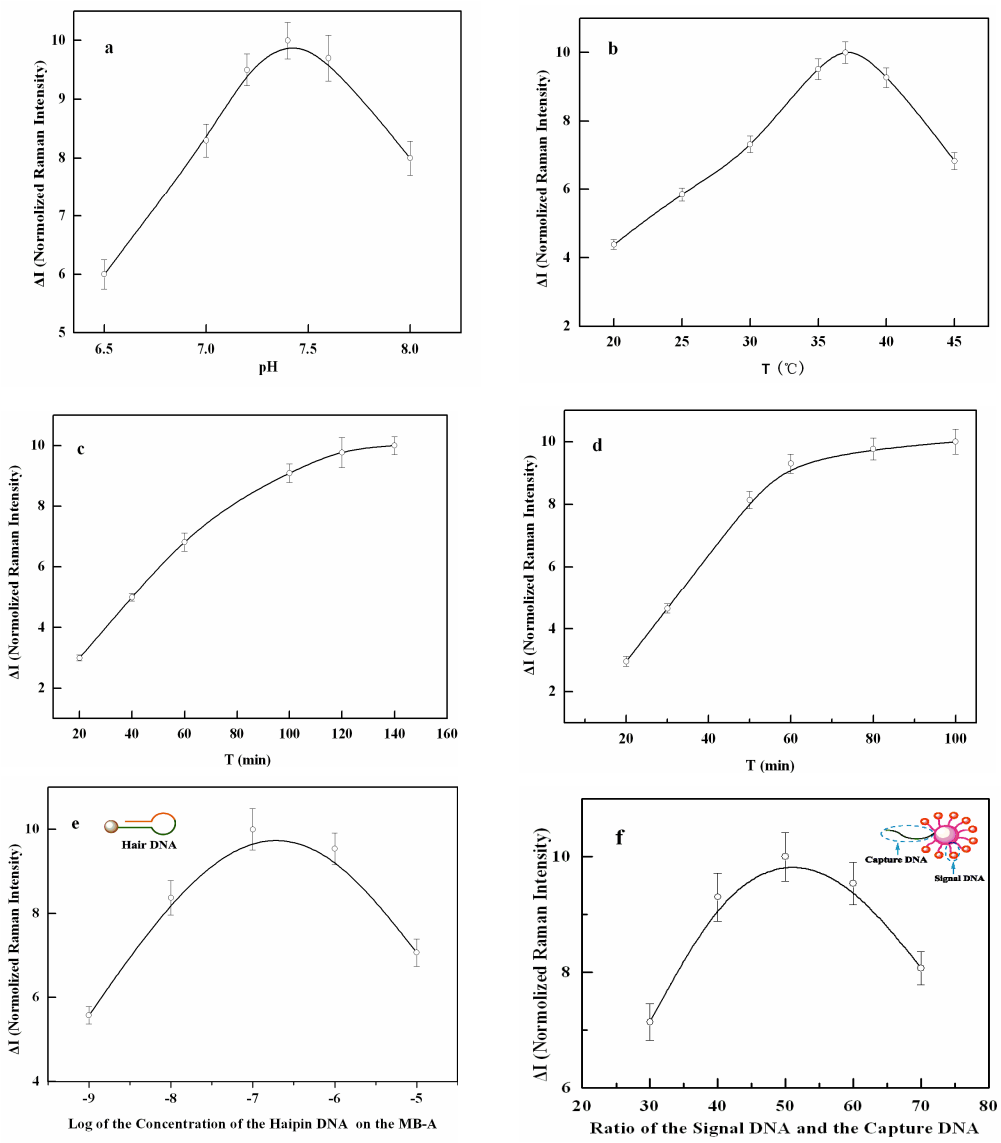

Figure S3. (a). Effect of the temperature and (b) pH of the cycle reaction. on the ΔI signal. (c). Effect of optimization of the time of the DNA machine operation and (d) RCA reaction on the ΔI signal. (e). Effect of the concentration of the hairpin DNA immobilized on the MB on the ΔI signal. (f). Effect of the proportion of the signal probes and the capture probe immoblized on the Au NP on the ΔI signal. Target DNA1 concentration, 10^{-14}

M.

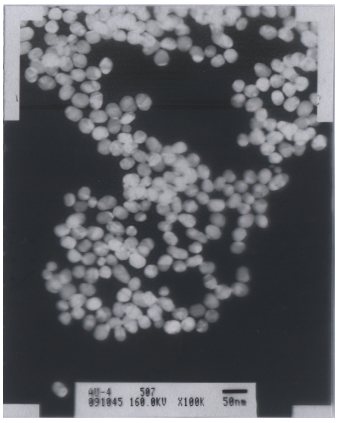

Figure S4.The TEM of Au NPs (~20 nm).

Selectivity for DNA detection.

The SERS intensity with probe A (non complementary DNA, column A), probe B (One base mismatched DNA-1, the base mismatched in the Nt. BbvCI recognition region, column B), probe C (One base mismatched DNA-2, column C), probe D (target DNA 1, column D) and target DNA 1 (in presence of 10^{-10} non complementary DNA, column E) were presented in the concentration respectively for 10^{-14} M, 10^{-13} M, 10^{-12} M. From Figure S5, it can be seen that in the presence of non complementary DNA or one-base mismatched DNA-1, no significant difference in SERS signals was observed as compared to the control group without target DNA 1, suggesting the scission machine is not activated towards the cutting of the hairpin loop region. The cycle 1 was not initiated, and DNA 3 as a RCA primer probe was not released, the RCA did not happened. Accordingly to the column C, the one-base mismatched DNA-2 produced a 60% lower analytical signal in comparison with the full matching target DNA in the presence of 10^{-12} M. In addition, No obvious difference in the SERS intensity of column D and column E, is suggesting the excess DNA had no significant effect on the detection of target DNA. All the analysis shows the present method has a good specificity for DNA detection.

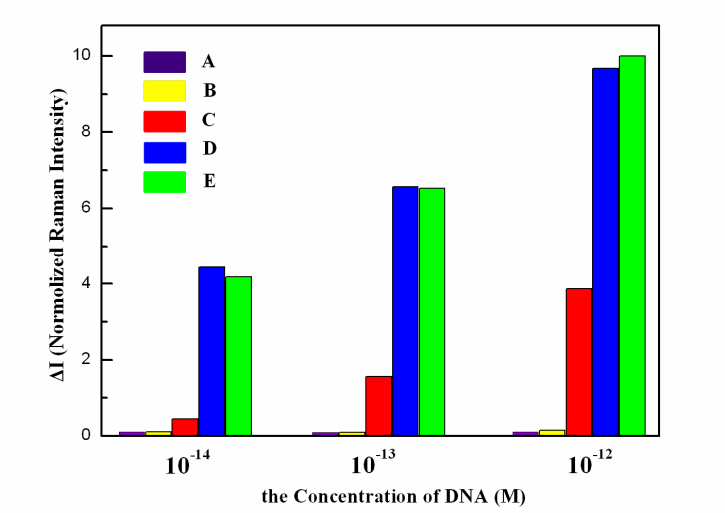

Figure S5. Specificity for the detection of DNA against non complementary DNA, one base mismatched DNA-1, one base mismatched DNA-2, target DNA 1 and target DNA 1 (in presence of 10^{-10} non complementary DNA), in the concentration respectively for 10^{-14} M, 10^{-13} M, 10^{-12} M. Normalized Raman intensity in presence of non complementary DNA, column A, one base mismatched DNA-1, colunmn B, one base mismatched DNA-2, column C, target DNA 1 column D and target DNA 1 (in presence of 10^{-10} non complementary DNA), column E. The blank was subtracted for each value.

Selectivity for the tumor cells detection. To assess the selectivity for the tumor cell detection, the experiments were conducted on HeLa and CEM cells. Ramaos cell and two control analytes of CEM and Hela with the same number were introduced into this network using the same method. As show in the figure S6, the presence of Hela17 and CEM cells led to a SERS signal equivalent to the blank sample, indicating that the unspecific cells barely generated signal on this sensor. The signal for the Ramos cells was much larger and apparently distinguishable from the SERS intensity of the above, the experimental results demonstrated that the proposed system for Ramos cells exhibited a good specificity originating from the specific binding between target and aptamer.

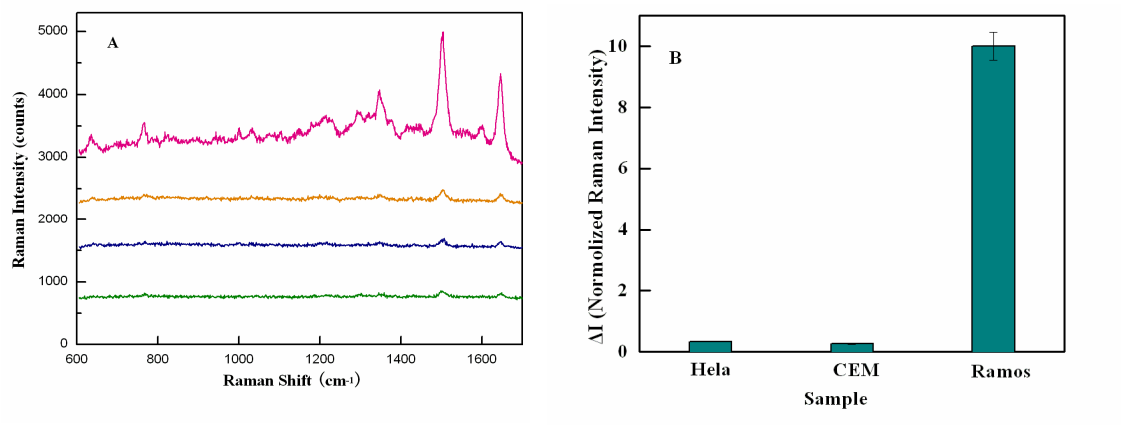

Figure S6. Specificity for the detection of Ramos cell against CEM and Hela. Numbers of Hela, CEM and Ramos cells all 50 cells, SERS spectra (A) and (B) normalized Raman intensity. The blank was subtracted for each value.

Real blood sample detection. Finally, the performance of the cell assay in actual serum was tested. Ramos cells were added in 20% human serum from healthy adults to known contents to simulate real blood samples and excess MCF-7 cells (the proportion of the number of Ramos cells to MCF-7 cells, 1: 100). The samples were then detected with the assay in this study, and the ratio of the measured value against added content was calculated as recovery rate. As shown in table S2, this recovery was 87%to 90%, implying the robustness of this assay in its application for real samples.

Table S2. Detection of adenosine in human serum

Nos.	Cell content detected (mL^{-1})	Ramos cell content added (mL^{-1})	MCF-7 cell Content (mL^{-1})	Cell content detected (mL^{-1})	recovery (%)
1	0	50	5000	45	90%
2	0	100	10000	87	87%
3	0	500	50000	436	87%

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