Electronic Supplementary Information

A highly sensitive surface plasmon resonance sensor for the detection of DNA and cancer cells by a target-triggered multiple signal amplification strategy

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Experimental Section

Reagents. All oligonucleotides used in the present study were synthesized and purified by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (China), and the sequences were listed in Table S1. Klenow fragment of E. coli DNA polymerase I (5 U μL^{-1}, denoted as "polymerase" for short) and the mixture of four dNTPs (2.5 mM for each component) were purchased from TaKaRa Bio Inc (Dalian, China). T4 ligase (5 U mL^{-1}) and the endonuclease (Nb.BbvCI, 10000 U mL^{-1}) were purchased from the NEW ENGLAND Biolabs (NEB). Hydrogen tetrachloroaurate (III) tetrhydrate (HAuCl4•4H2O), trisodium citrate, hydrochloride (EDC) were ordered from Sigma-Aldrich (America). Other chemicals employed were of analytical reagent grade and were used without further purification. The carboxy-modified magnetic nanoparticles (COOH-MNPs; size: 100-500 nm) were purchased from Tianjin BaseLine ChroTechResearch Centre (China). Doubly distilled water was used throughout the experiments. The gold chips used for SPR detection were purchased from BioNavis Ltd (Finland).

Table S1. DNA sequences used in this work.

Name	Sequences	Discription
S0	5'-AACACCGTGGAGGATAGTTCGGTGGCTGTTCAGGGTCTCCTCCCGGTGAAAAA-NH_2-3'	Romas cell aptamer DNA
S1	5'-GGCCTCCACGGTGTTGTCGCT-3'	Target DNA, partial complementary with 5'-end of aptamer DNA and complementary with S2
S2	5'-SH- TTTTTTTTGGGCGGGCGGTGCATGCGACAACACCGTGGAGGCCTCAGCCACCGCCCGCCCTT - 3'	Hairpin DNA immobilized on the gold substrate
S3	5'-NH_2- TTTTTTTCTTCTCTTTGGGCGGGCGG-3'	Complementary with 3'-end of DNA S2 and immobilized on the MNP
S4	5'-NH_2- TTTTCGAGTCAGAGGTGTGGAGTC-3'	RCA primer DNA
S5	5′-PO_4-CTCTGACTCGTCGTTCGCCGTTCTAGCAAAATCGTTCGCCGTTCTAGCGACTCCACAC-3'	Padlock probe
S6	5′-SH-TTTTTTCGTTCGCCGTTCTAGC-3′	Complementary with RCA product
S7	5′-SH-TTTTTTCGAGGTTTTT-3′	Noncomplementary DNA as bio-bar-codes to avoid cross -reaction
S8	5'- GGCCTCCAC *T* GTGTTGTCGCT-3'	Single base-mismatched DNA

S9	5’-GGCCT *T* CACGGT *T* TTGTCGCT-3’	Two base-mismatched DNA
S10	5’-*CCAACTTTATTCAGGAGAATG*-3’	Noncomplementary DNA

Apparatus. SPR detection was performed on a SPRNavi200 (BioNavis, Finland). UV/Vis absorption spectra were obtained with a Cary 50 Series Spectrophotometer (Varian, Australia). Transmission electron microscopy (TEM) imaging was taken with JEM-2000EX/ASID2 instrument (Hitachi, Japan). Scanning electron microscopy (SEM) imaging was performed by a JEOL JSM-6700F instrument (Tokoy, Japan).

Preparation of Au NPs and Au NPs bio-barcode probe

Gold nanoparticles (AuNPs) were synthesized following the previously reported method by reduction of tetrachloroauric acid ($HAuCl_4$) with sodium citrate.[1] The prepared gold nanoparticles were stored in brown glass bottles at 4 °C and characterized by TEM (Figure S1). The Au NP bio-barcode probe was obtained by capping the DNA S7 and DNA S6 on the surface of AuNPs. The process of Au NP bio-barcode probe according a literature[2, 3] was enforced as follows: the 10^{-6} M DNA S7 and 10^{-7} M DNA S6 with the molar ratios of 20:1[4] 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 resulting Au NPs bio-barcode probes were dispersed into a PBS buffer solution (0.01 mM, pH 7.4) and stored at 4 °C for further use. The successful immobilization of DNA on the AuNP surface could be observed from the UV-vis absorbance difference between the AuNPs solution before immobilization and the DNA-AuNPs solution after immobilization.

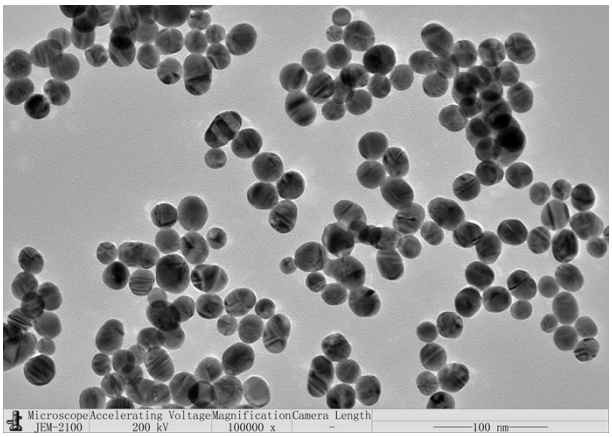

Figure S1. TEM images of AuNPs

Immobilization of DNA onto MNPs

The process of the immobilization of DNA onto MNPs according a literature[5] was enforced as follows: Carboxy-modified MNPs were washed three times with imidazole-HCl solution (0.1 M, pH 6.8), then were washed by PBS (10μL, 0.1 M, PH 7.4) three times. MNPs (5μL) and EDC (200μL, 0.1M) mixture solution were incubated for 30 min and shaked gently in 37 **°C** to activate the carboxyl groups on the MNPs. Finally, Romas cell aptamer DNA (10μL, 10^{-7} M) or the mixture of DNA S4 and S3 with different molar ratios was separately added into the activated MNPs (MNP-A, MNP-B), the resulting mixture was incubated at 37 **°C** for 12 h with gentle shaking. The DNA-modified MNPs and MNP bio-bar-code probe were rinsed three times with PBS (200 μL, 0.01 M, pH 7.4), and magnetically separated. Then the conjugates were dispersed in the PBS solution before used.

Preparation of hairpin probe-modified Substrate

Before the modification of hairpin aptamer probe, the gold chip was immersed in a boiling solution (30% H_2O_2, 28% ammonia, and double distilled water in a volume ratio of 1:1:5) for 10 min. Then the cleaned chip was rinsed thoroughly with double distilled water, and dried by nitrogen gas prior to use. Meanwhile, the poly(dimethylsiloxane) PDMS film with the empty hole was cleaned in the ethyl alcohol by the ultrasonic cleaner for a three minutes then rinsed by pure water and dried by nitrogen. The DNA S2 was first incubated at 95 °C for 10 min then cooled slowly to room temperature to ensure a hairpin structure. After the PDMS film was immobilized onto the chip, 30 μL of 5.0×10^{-7} M DNA S2 was injected into the reaction tank assembled on the gold chip, and incubated for 15-30 h in a humidity chamber. Subsequently, the 6-mercapto-1-hexanol (MCH) was added to the holes to block the nonspecific binding sites for 2h. Finally, the gold chips were successively rinsed with D.I. water and PBS solution to remove free and excess DNA.

Multiple isothermal amplification reactions

After the hairpin probe S2 was immobilized onto the gold chip, different concentrations of target DNA S1, Klenow polymerase (0.2 U/μL), Nb.BbvCI (0.4 U/μL), dNTPs (5.5 μL, 1 mM), MNP bio-bar-code probe were added into the above-mentioned reaction tank assembled on the gold chip to perform the target DNA cycle exponential amplification reaction. After incubated at 37 °C for 100 min, the

MNP bio-bar-code probe connected with gold chip and the chip was washed three times with PBS buffer subsequently. Then, padlock probe S5 and T4 DNA ligase were added in the reaction tank. This complex was incubated at 37 °C. Subsequently, the RCA reaction was carried out with the addition of 5 μL 10 mM dNTPs, 2 μL 10 U/μL Klenow polymerase at 37 °C for 60 min. After RCA reaction, the reaction tank was removed from gold chip and the chip was washed three times with PBS buffer.

SPR Online Assay

The SPR measurements were conducted using the SPRNavi200 (BioNavis, The Finland), a double-channel and prism coupling-based instrument. After multiple amplification reactions, the obtained gold chip was immobilized on the flow cell and washed by flowing PBS buffer (50 μL min^{-1} flow rate) until the baseline was constant. The bio-bar-code AuNPs were injected and hybridized with the RCA products. In a kinetic measurement mode, molecular adsorption on gold chip was followed by monitoring SPR angle shifts (θ) or angle changes ($\Delta\ \theta$) over time. Finally, the substrate was washed with buffer and the difference in the response singnal was computed by taking the difference between the initial and final buffer signals.

Analysis of Romas cell

The aptamer of Romas cell (0.1 μM) was immobilized on the surface of MNPs and hybridized with the complementary sequence (S1, 0.1 μM) to form the duplex structure. Different concentrations of Romas cell were added to this system, and shaken gently at 37 °C for 30 min, magnetically separated. The released DNA S1 was in the supernatant. Then, the released DNA S1 acted as target DNA to enter into the DNA amplification system and triggered the multiple isothermal amplification reactions. The following processes were the same as the target DNA assay.

Characterization of Au NPs bio-barcode probe

The prepared Au NPs bio-barcode probes were characterized by UV-visible spectra. As shown in Figure S2, Curve a, b respectively exhibited the characteristic absorbance of DNA S6, S7 at ~260 nm. Curve d exhibited the characteristic absorbance of gold nanoparticles (AuNPs) at ~520 nm. Curve c exhibited both the characteristic absorbance of DNA and the characteristic absorbance of AuNPs, which indicated that the AuNPs were successfully labeled with DNA.

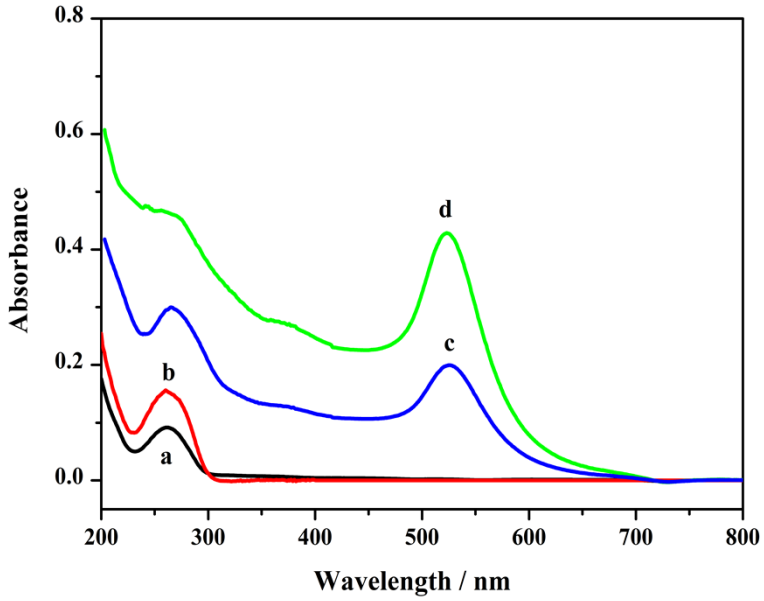

Figure S2. UV spectra of DNA S6 (a), DNA S7 (b), Au NPs bio-barcode probe (c), and AuNPs (d).

Optimization of Assay Parameters

Optimization of the pH for the isothermal amplification reactions. Taking account of the amplification efficiency for DNA assay, the effect of pH of the PBS buffer solution for the isothermal amplification reaction was investigated in the pH range from 6.5 to 8.5. As shown in Figure S3, the resonance angle changes reached the maximum at pH 7.4. Thus, the pH 7.4 was used as an optimal condition.

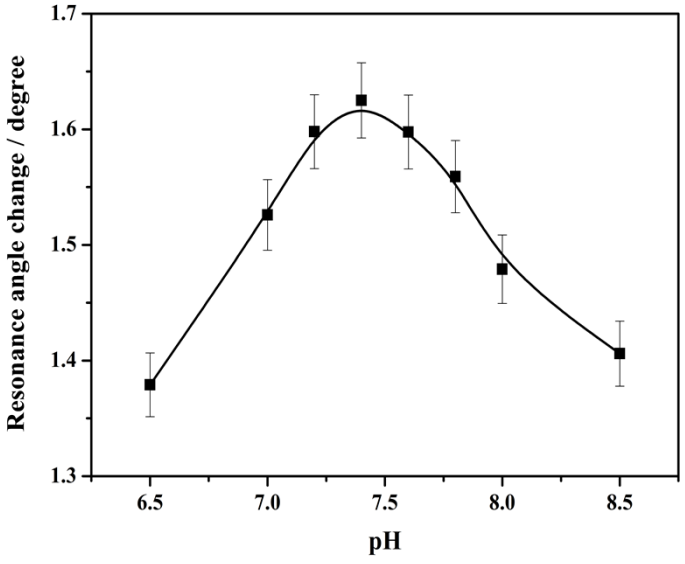

Figure S3. Influence of pH for the isothermal amplification reactions.

Optimization of the temperature. The temperature had a crucial effect on the reactivity of enzyme and the DNA hybridization efficiency. Figure S4 showed the influence of the temperature on the resonance angle changes produced by 1.0×10^{-15} M target DNA. The maximal resonance angle change was obtained at 37 °C. Thus, 37 °C was employed as the optimal experimental temperature, which was consistent with the fact that enzymatic reactions are usually operated at 37 °C due to the best bioactivity of enzymes.

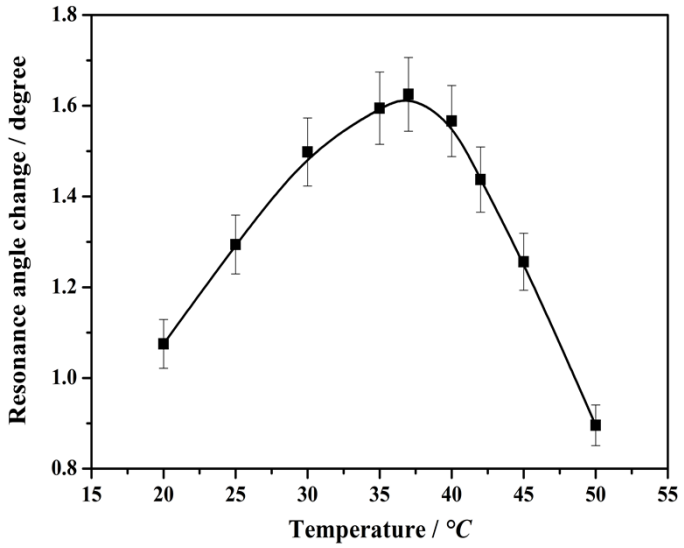

Figure S4. Influence of reaction temperature for the isothermal amplification reactions responding to 1.0×10^{-15} M target DNA.

Optimization of the proportion of the bio-barcode probe immobilized on the MNPs. The bio-barcode MNPs are functionalized with DNA S3 to anneal with the open stem of hairpin probe and DNA S4 to hybridize with RCA templates. The resonance angle changes was also influenced by the proportion of the DNA S4 and the DNA S3 immobilized on the MNPs. To improve the sensitivity of SPR quantification of target DNA, the proportion of the DNA S4 and the DNA S3 was optimized. Figure S5 shows the variance of resonance angle changes with the proportion of the DNA S4 and the DNA S3. It was obviously that signal increased upon raising the proportion from 10:1 to 30:1, and reached the maximum at 30:1. Thus the ratio of 30:1 was selected for the subsequent assays.

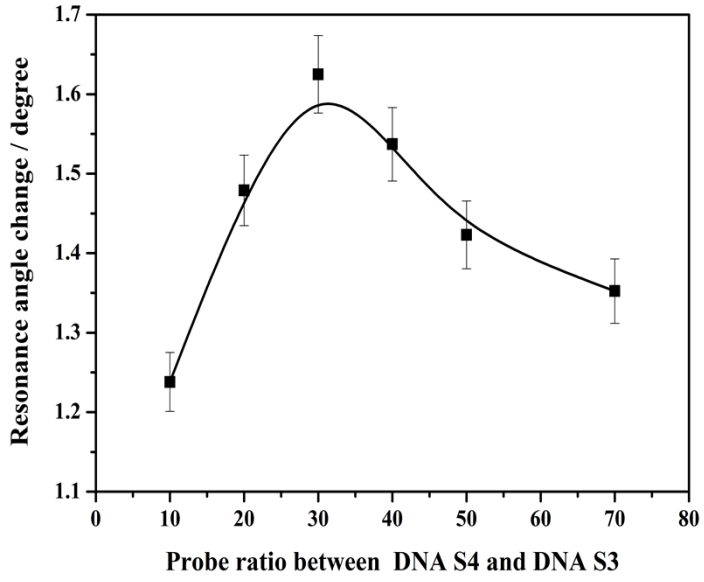

Figure S5. Resonance angle changes for different ratio of DNA S4 to DNA S3.

Optimization of the size of AuNPs. To improve the sensitivity of SPR quantification of taget DNA, the size of the AuNPs was optimized. In this work, Au NPs with average diameters of 12, 16, 25, 40, 60, and 70 nm, respectively, were selected. As shown in Figure S6, the SPR signal increased with the increase of AuNPs size from 12 to 25 nm, and reached the maximum at 25 nm. These results demonstrated that the oligonucleotide amount decreased with increasing AuNPs size,[3] but the large gold particles were easy to aggregate, and might increase the steric hindrance. Thus the size of 25 nm was selected for the subsequent assays.

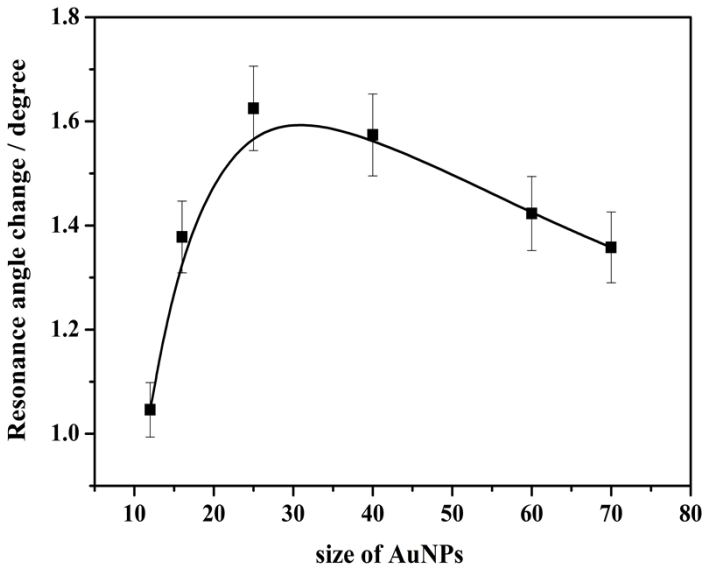

Figure S6. SPR signals for different size of AuNPs. The concentration of DNA is 1.0×10^{-15} M.

Optimization of the time of amplification reaction. In cascade DNA amplification system, the upstream T-EXPAR and the downstream RCA reaction coexisted in this system to amplify signals. The time of two reactions were investigated, respectively. As shown in Figure S7, when the reaction time increased from 10 to 100 min, the resonance angle changes increased gradually. After that, the signal tended to the platform. Therefore, 100 min was considered to be the optimum time used in the T-EXPAR amplification reaction. And 60 min was chosen as the optimal reaction time for RCA (see Figure S8).

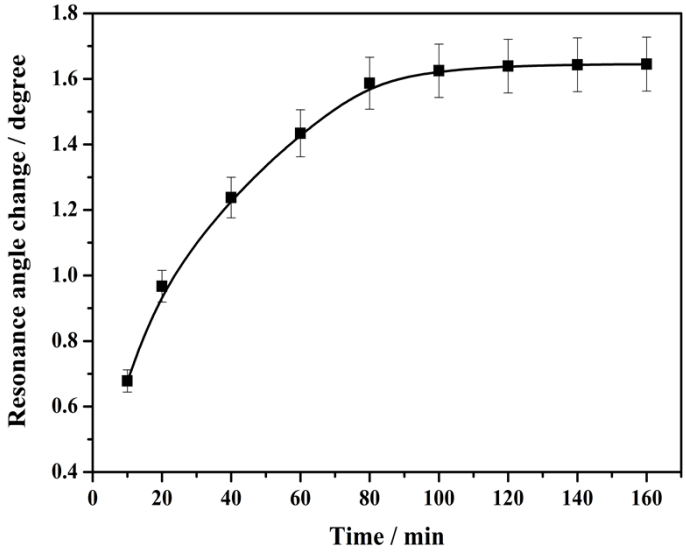

Figure S7 Influence of the T-EXPAR reaction time on the SPR signal.

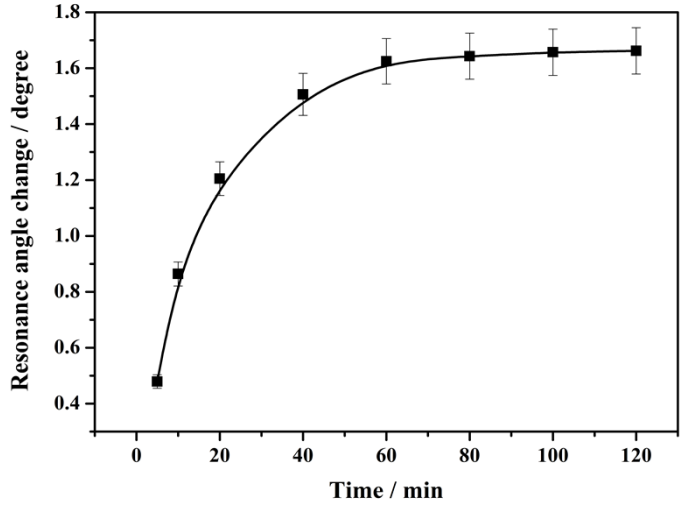

Figure S8 Influence of the RCA reaction time on the SPR signal responding to 1.0×10^{-15} M target DNA.

Optimization of the concentration of the hairpin probe immobilized on the gold chip. The hairpin probe immobilized on the gold film surface was used as the capture probe. The high concentration of hairpin structured probes might increase steric hindrance for the target binding, leading to a low signal gain.[6] On the other hand, low concentration could not bind sufficient target. Figure S9 showed the variance of SPR singnal with the concentration of capture probe. In the presence of 1.0×10^{-15} M DNA, the SPR signal increased with the increase of probe concentration from 5.0×10^{-8} M to 5.0×10^{-7} M, reached a maximum value and then decreased monotonically beyond the concentration of 5.0×10^{-7} M. Therefore, the concentration hairpin probes of 5.0×10^{-7} M was employed in the following work.

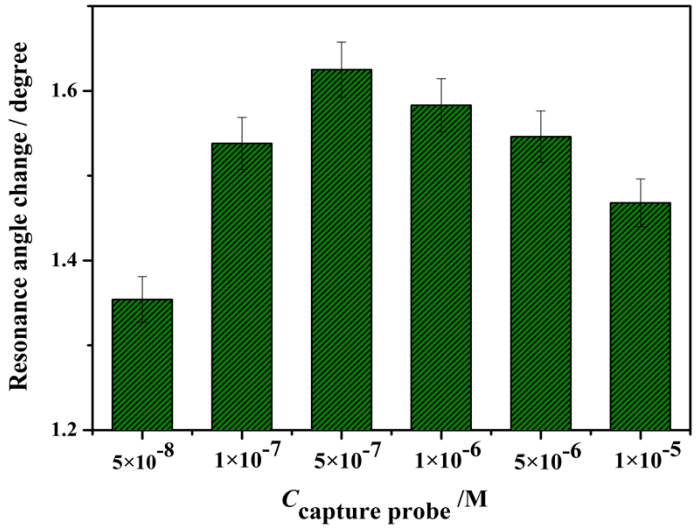

Figure S9. Effect of the concentration of the capture probe on the SPR signal.

The Comparison of Different Amplification Formats for Detection of the Target DNA

Detection format a: AuNP amplification

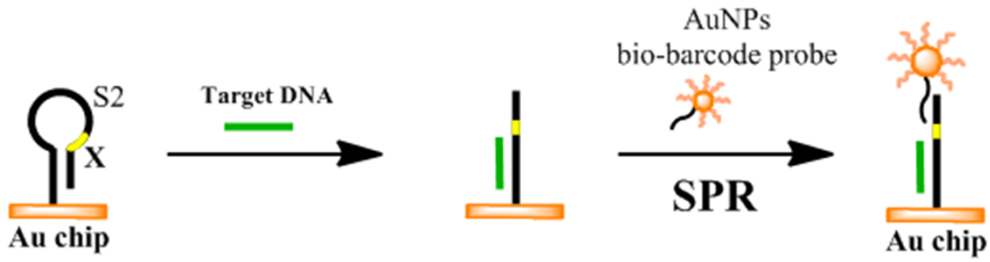

Figure S10. Schematic representation of the AuNP-enhanced SPR assay.

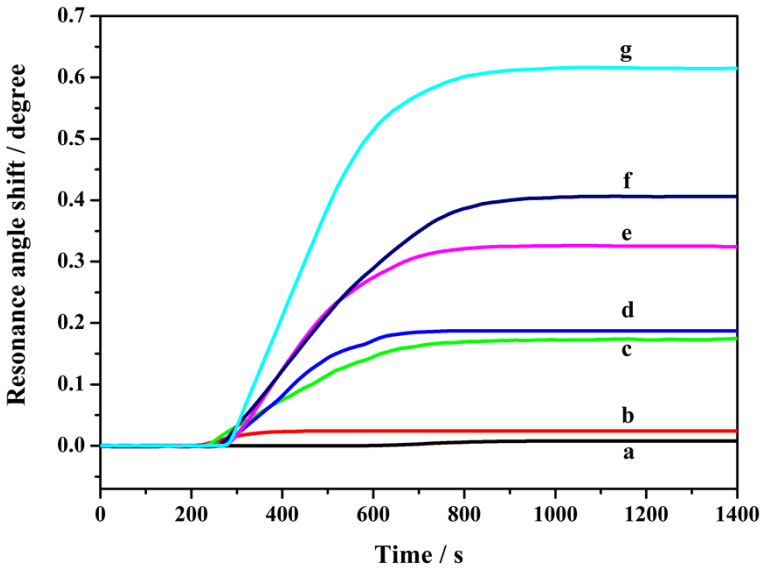

Figure S11. Real-time resonance angle responses of the AuNP-enhanced SPR assay for DNA detection. From a to g: 0, 1.0×10^{-11}, 5.0×10^{-11}, 1.0×10^{-10}, 5.0×10^{-10}, 1.0×10^{-9}, 1.0×10^{-8} M DNA.

Detection format b: AuNP-RCA amplification

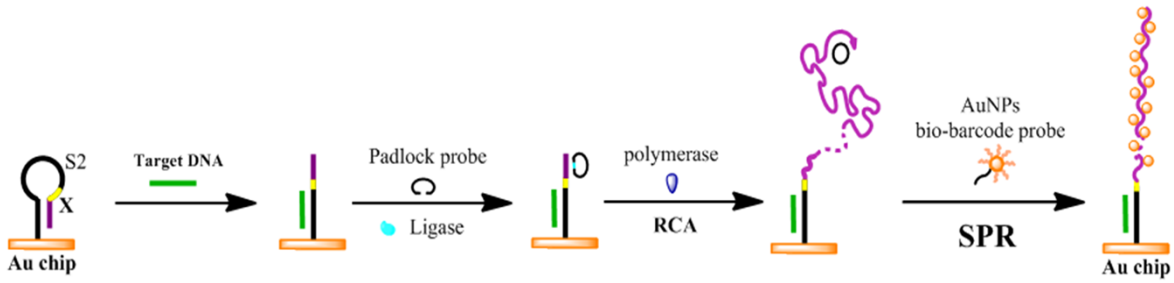

Figure S12. Schematic representation of the AuNP-RCA SPR assay.

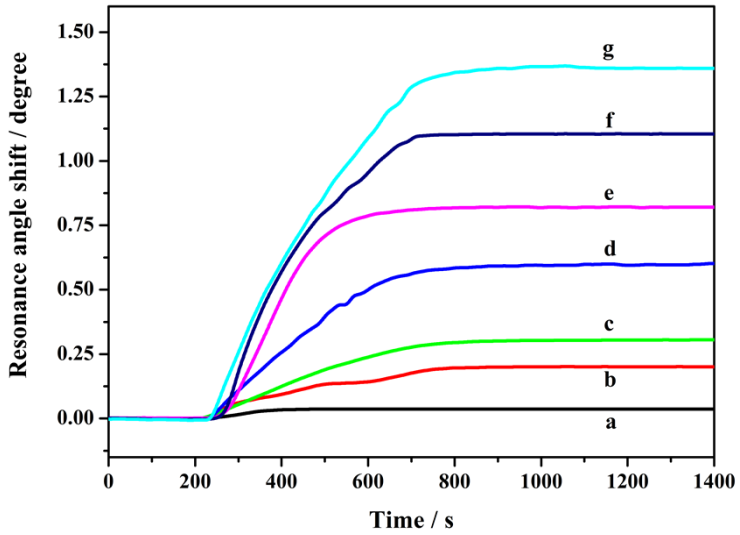

Figure S13. Real-time resonance angle responses of the AuNP-RCA SPR assay for DNA detection. From a to g: 0, 5.0×10^{-13}, 1.0×10^{-12}, 5.0×10^{-12}, 1.0×10^{-11}, 5.0×10^{-11}, 1.0×10^{-10} M DNA.

Detection format c: AuNP-MNP-RCA amplification

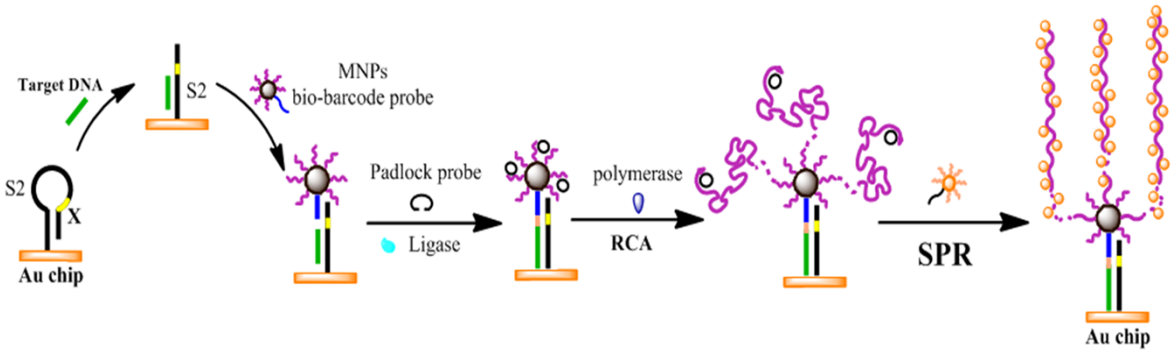

Figure S14. Schematic representation of the AuNP-MNP-RCA SPR assay.

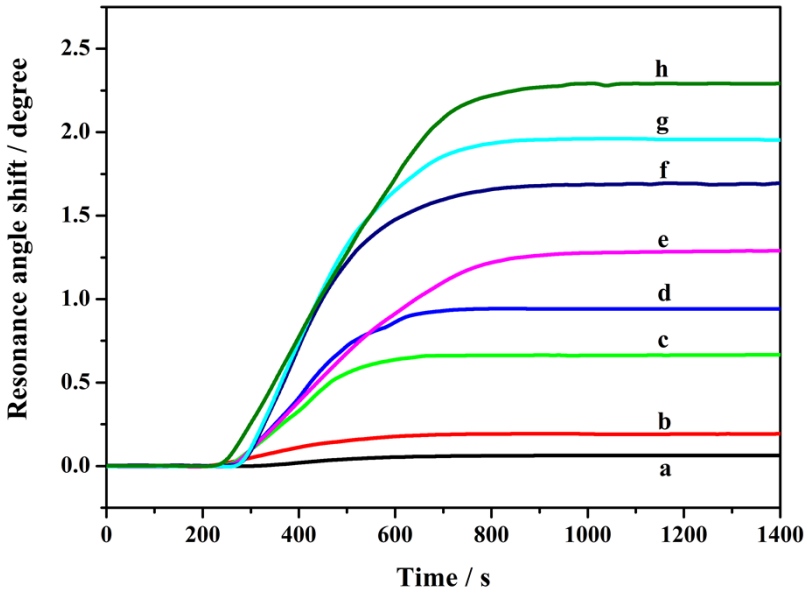

Figure S15. Real-time resonance angle responses of the AuNP-MNP-RCA SPR assay for DNA detection. From a to h: 0, 5.0×10^{-14}, 1.0×10^{-13}, 5.0×10^{-13}, 1.0×10^{-12}, 5.0×10^{-12}, 1.0×10^{-11}, 5.0×10^{-11} M DNA.

Detection format d: AuNP-SPD-MNP-RCA amplification

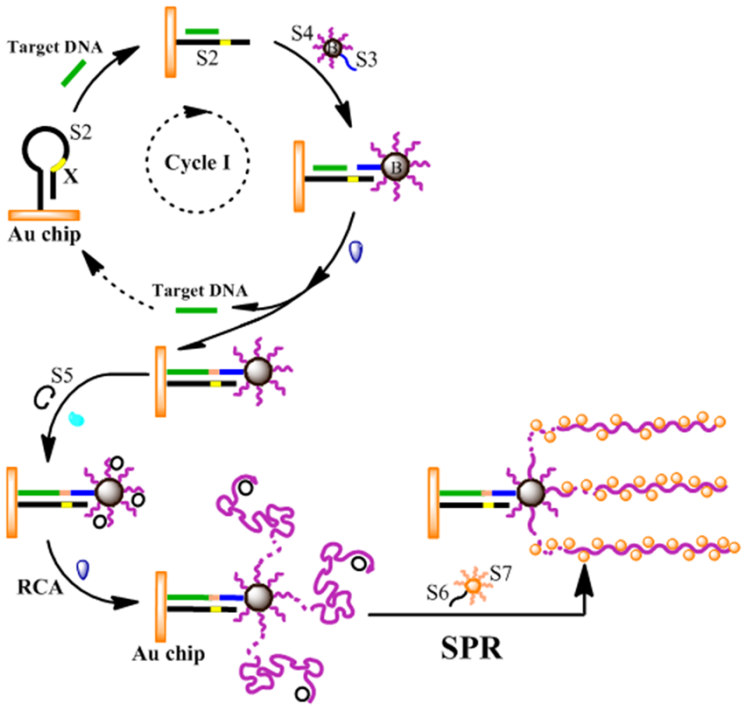

Figure S16. Schematic representation of the AuNP-SPD-MNP-RCA SPR assay.

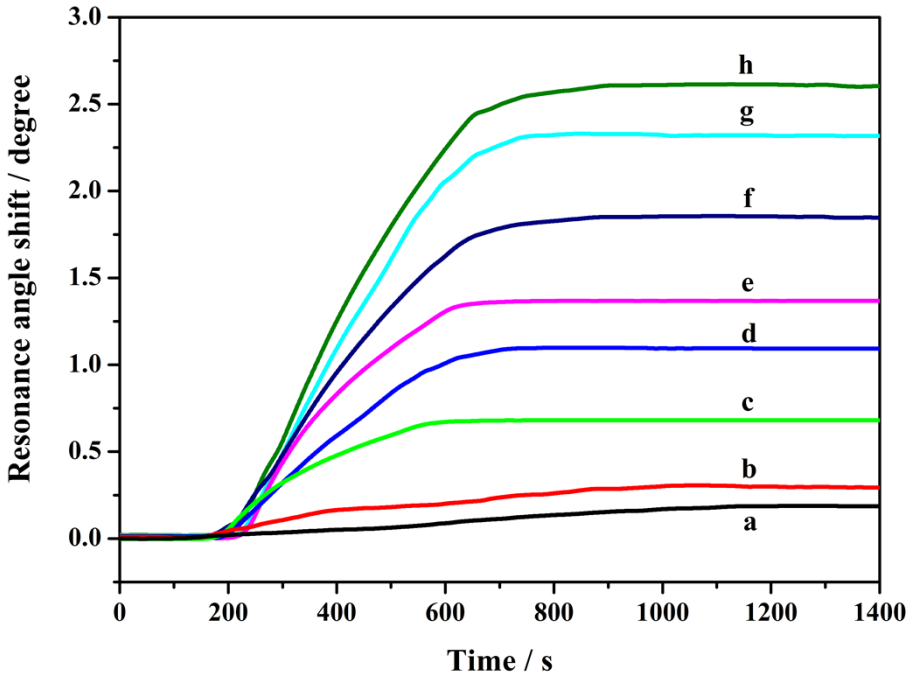

Figure S17. Real-time resonance angle responses of the AuNP-SPD-MNP-RCA SPR assay for DNA detection. From a to h: 0, 1.0×10^{-15}, 5.0×10^{-15}, 1.0×10^{-14}, 5.0×10^{-14}, 1.0×10^{-13}, 5.0×10^{-13}, 1.0×10^{-12} M DNA.

Comparison of sensitivity among the method involved in our research

Table S2. The sensitivity of five amplification formats.

Nos.	Amplification format	Sensitivity	Sensitivity improvement compared to AuNP-enhanced
a	AuNP-enhanced	1.0×10^{-11} M	-
b	AuNP-RCA	3.5×10^{-13} M	29 times
c	AuNP-MNP-RCA	4.5×10^{-14} M	2.2×10^{2} times
d	AuNP-SPD-MNP-RCA	1.0×10^{-15} M	1.0×10^{4} times
e	AuNP-EXPAR-MNP-RCA	9.3×10^{-18} M	1.1×10^{6} times

Comparison of different methods for DNA detection.

Table S3. Total assay time and detection limit comparison between our method and other reported ones

Assay	Indicator or amplification	Total assay time	Detection limit	ref
surface plasmon resonance	polymerase chain reaction (PCR)	100 min	0.15 μM	7
electrochemical detection	PCR	100 min	80 nM	8
surface enhanced Raman scattering	PCR	120 min	1 nM	9
surface plasmon resonance imaging	enzyme exonuclease III in conjunction with DNA microarrays	60 min	10 pM	10
Scanning Electrochemical Microscopy	DNA Microarrays Enhanced by HRP-Modified SiO_2 Nanoparticles	162 min	1pM	11
surface plasmon resonance imaging	DNA microarray and surface-based RNA transcription	132 min	1 fM	12
surface enhanced Raman scattering	rolling circle amplification(RCA)	120 min	10 pM	13
electrochemical detection	autocatalytic and exonuclease III (Exo III)-assisted target recycling amplification	150 min	10 fM	14
fluorescence detection	hybridization chain reaction	100 min	0.675 fM	15
chemiluminescent detection	exonuclease III-assisted target recycling amplification and catalytic effect of	150 min	12 fM	16

	G-quadruplex-hemin DNAzyme			
colorimetric DNA assay	Gold nanoparticle-enabled real-time ligation chain reaction	90 min	20 aM	17
electrochemical detection	Template enhanced hybridization process (TEHP) and RCA	120 min	11 aM	18
colorimetric detection	gold nanoparticles and hybridization chain reaction amplification	60 min	50 pM	19
electrochemiluminescence detection	Au nanoparticles and isothermal circular double-assisted amplification	40 min	5 aM	20
surface enhanced Raman scattering	target-triggering cascade cycle amplif-ication	90 min	72 aM	21
electrochemical detection	Exo III-aided autocatalytic target recycling	30 min	0.1 pM	22
electrochemical detection	enzyme-assisted target recycling and DNA supersandwich assemblies	180 min	0.36 fM	23
quartz crystal microbalance	self-assembled DNA nanostructure	225 min	0.1 nM	24
surface plasmon resonance	target-triggering isothermal exponent-tial amplification with the magnetic nanoparticle-based RCA	165 min	9.3 aM	this work

Specificity of Ramos Cells Detection

The specificity of this assay was investigated by detecting 0 Ramos cells (blank), 5000 MCF-7 cells (control), and 500 Ramos cells (target), respectively. The highest SPR signal was observed in the presence of target Ramos cells, while weak signal was observed in the absence of Ramos cells or in the presence of MCF-7 cells (Fig. 3B), demonstrating that the designed strategy was specific for the target cell. The SPR signals obtained after treatment with Ramos cells and mix cells in buffer and real blood sample (RBS) did not show significant difference (Fig. 3B), indicating very little interference of complex media on the designed strategy.

Detection of Ramos Cells in Human Blood

Table S4. SPR signal of blood samples spiked with Romas target cells

Sample	Added target cell (cell/mL)	Detected cell (cell/mL)	Recovery (%)	RSD (%)
1	50	46	92%	7.3
2	200	183	91.5%	4.9

3	2000	1956	97.8%	8.2

Each sample was repeated for three times and averaged to obtain the recovery and RSD values.

Reference

[1] G. Frens, *Nature Physical Science*, 1973, **241**, 20.

[2] T. A. Taton, C. A. Mirkin, R. L. Letsinger, *Science*, 2000**, 289**, 1757.

[3] S. J. Hurst, A. K. R. Lytton-Jean, C. A. Mirkin, *Anal. Chem.* 2006, **78**, 8313.

[4] P. He, L. J. Liu, W. P. Qiao, S. S. Zhang, *Chem. Commun.*, 2014, **50**, 1481.

[5] J. Miao, Z. Cao, Y. Zhou, C. Lau, J. Lu, *Anal. Chem*,. 2008, **80**, 1606.

[6] Y. Cao, S. Zhu, J. Yu, X. Zhu,Y. Yin, G. Li, *Anal. Chem.* 2012, **84**, 4314.

[7] E. Kai, S. Sawata, K. Ikebukuro, T. Iida, T. Honda, I. Karube, *Anal. Chem.*, 1999, **71**, 796.

[8] O. Dilsat, T. Burcin, K. Buket, O. Mehmet, *Anal. Chem.*, 2008, **80**, 588.

[9] V. Danny, F. Karen, G. Duncan, *Anal. Chem.*, 2011, **83**, 5817.

[10] H. J. Lee, Y. Li, A. W. Wark, and R. M. Corn, *Anal. Chem.*, 2005, **77**, 5096.

[11] H. Fan, X. Wang, F. Jiao, F. Zhang, Q. Wang, P. He, Y. Fang, *Anal. Chem.*, 2013, **85**, 6511.

[12] I. E. Sendroiu, L.K. Gifford, A. Lupták, R. M. Corn, *J. Am. Chem. Soc.*, 2011, **133**, 4271.

[13] J. Hu, C. Zhang, *Anal. Chem.*, 2010, **82**, 8991.

[14] S. Liu, C. Wang, C. Zhang, Y. Wang, B. Tang, *Anal. Chem.*, 2013, **85**, 2282.

[15] Y. Liu, M. Luo, J. Yan, X. Xiang, X. Ji, G. Zhou, Z. He, *Chem. Commun.*, 2013, **49**, 7424.

[16] Y. Gao ,B. Li, *Anal. Chem.*, 2013, **85**, 11494.

[17] W. Shen, H. Deng, Z. Gao, *J. Am. Chem. Soc.*, 2012, **134**, 14678.

[18] H. Ji, F. Yan, J. Lei, H. Ju, *Anal. Chem.*, 2012, **84**, 7166.

[19] P. Liu, X. Yang, S. Sun, Q. Wang, K. Wang, J. Huang, J. Liu, L. He, *Anal. Chem.*, 2013, **85**, 7689.

[20] H. Zhou, J. Liu, J. Xu, H. Chen, *Chem. Commun.*, 2011, **47**, 8358.

[21] P. He, Y. Zhang, L. Liu, W. Qiao, S. Zhang, *Chem. Eur. J.*, 2013, **19**, 7452.

[22] S. Liu, Y. Lin, L. Wang, T. Liu, C. Cheng, W. Wei, B. Tang, *Anal. Chem.*, 2014, **86**, 4008.

[23] Y. Chen, Q. Wang, J. Xu, Y. Xiang, R. Yuan, Y. Chai, *Chem. Commun.*, 2013, **49**, 2052.

[24] W. Tang, D. Wang, Y. Xu, N. Li, F. Liu, *Chem. Commun.*, 2012, **48**, 6678.