Supplementary Information

Colorimetric sensing platform based upon recognizing hybridization chain reaction product with oligonucleotide modified gold nanoparticles through triplex formation

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Experimental Section

Materials

All DNA oligonucleotides used in this study (Table) were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China) and the sequences were listed in Table S2. Chloroauric acid ($HAuCl_4 \cdot 4H_2O$) and tris (2-carboxyethyl) phosphine hydrochloride were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Trisodium citrate and spermine ($H_2N(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$) were obtained from Aladdin Reagent (Shanghai, China). Ultrapure water (18.2 MΩ·cm) obtained from a Milli-Q water purification system (Millipore Co., USA) was used in all experiments. Concentrated DNA stock solutions were prepared in a buffer and stored in a fridge at 4 °C. Other reagents were all of analytical reagent grade and used as received.

Apparatus

All absorption spectra were recorded on a UV-1750 UV-vis spectrophotometer (Shimadzu, Japan) at room temperature. Transmission electron microscopy (TEM) measurements were performed on a JEM-3010 transmission electron microscope. The samples for TEM characterization were prepared by dropping colloidal solution onto a carbon-coated copper grid and drying at room temperature. Dynamic light scattering (DLS) tests were performed using a Nano ZS/Mastersizer 2000E (Malvern Instruments Ltd., Malvern, UK) at room temperature. The images of gel electrophoresis were scanned by the Gel Image Analysis System (JY02S, Beijing, China). Circular dichroism (CD) spectroscopy was carried out using a J-810-150S spectropolarimeter (JASCO International CO. Ltd, Japan).

Preparation of DNA-functionalized Gold Nanoparticles

AuNPs were prepared according to the literature method with an appropriate modification.[1] A volume of 2 mL sodium citrate solution (38.8 mM) was added to the boiling HAuCl4 solution (1 mM) quickly. After the solution color changed from pale yellow to wine red, the reaction solution was allowed to stir for 10 min under

refluxing. Then, the solution was allowed to cool down to room temperature with continuous stirring, filtered through a 0.45 μm nylon filter and stored in a refrigerator at 4 °C before use. AuNPs functionalized with thiol-modified DNA were prepared according to the method proposed by Mirkin et al.[2] Thiolated DNA was treated with TCEP beforehand, subsequently, mixed with AuNPs in a 200:1 molar ratio for 16 h. Then, the mixture was aged in following 44 h along with addition of NaCl (the final concentration is 0.1 M). Finally, the solution was centrifuged three times (13800 rpm, 30 min), and the obtained oily precipitate was dissolved in 10 mM PBS solution (0.1 M NaCl, pH = 6.0) then stored at 4 °C for further use.

Detection procedure

Three DNA hairpin probes (HP, H1 and H2) were individually heated at 95 °C for 3 min and then gradually cooled to room temperature for 1 h before use. Target samples were mixed with HP (5.0 nM), H1 (100 nM) and H2 (100 nM) in the reaction buffer (10 mM PBS, 100 mM NaCl, pH = 6.0) and then were incubated at 37 °C for 3 h. Subsequently, 100 μL of the above reaction mixture was added into 100 μL of the prepared DNA-functionalized AuNPs solution. Finally, appropriate spermine was added into the mixture. After thorough shaking and incubating for about 8 h at room temperature, the color changes of the solutions and the corresponding UV-vis absorption spectra were recorded.

Gel electrophoresis analysis of the HCR products and triplex formation

Agarose gel electrophoresis was carried out to confirm the presence of the HCR event. Hairpin probes were separately heated at 95 °C for 3 min and then allowed cooled to room temperature for 1 h before use. Different mixtures of oligonucleotides were incubated at 37 °C for 24 h. The 2% agarose gels containing 4S Red Plus Nucleic Acid Stain (1 μL per 10 mL of gel volume) were prepared by using 1 × TBE buffer. The gel was run at 120 V for 1 h, visualized under UV light and finally photographed with a digital camera. The triplex formation was analyzed via

polyacrylamide gel electrophoresis (12%, 100 V, 90 min), stained with 4S Red Plus Nucleic Acid Stain and then visualized under UV light.

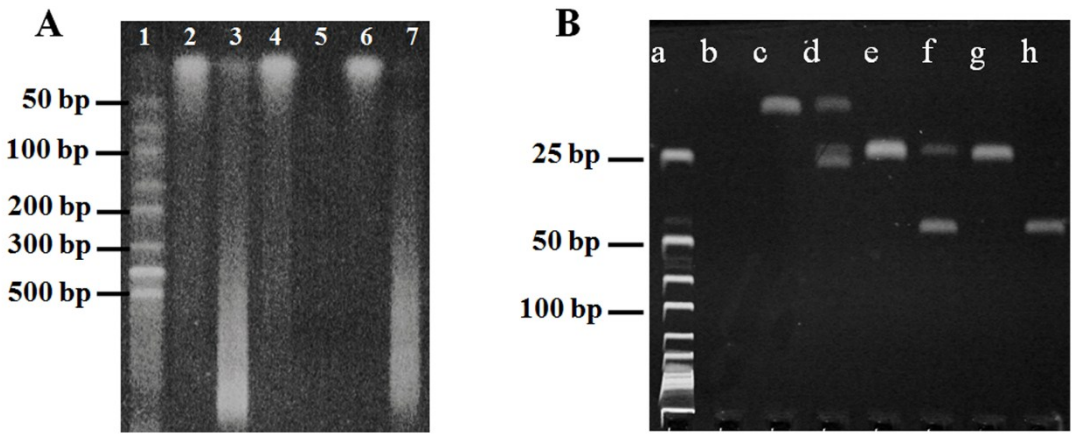

Fig. S1 (A) Agarose gel electrophoresis image of HCR products. Lane 1: the DNA marker; Lane 2: H1 + H2; Lane 3: H1 + H2 + Initiator; Lane 4: H1 + H2 + target; Lane 5: HP + target; Lane 6: H1 + H2 + HP; Lane 7: H1 + H2 + HP + target. The concentrations of H1, H2, HP and target were 1 μM, 1 μM, 50 nM and 50 nM. (B) Polyacrylamide gel electrophoresis image of triplex system. Lane a: the DNA marker; Lane b: T1; Lane c: T2 + T3; Lane d: T1 + T2 + T3; Lane e: H1; Lane f: H1 + TFO; Lane g: H2; Lane h: H2 + TFO. All DNA species were at 1 μM.

Circular dichroism (CD) spectroscopy

To demonstrate the formation of triplex DNA, circular dichroism spectroscopy of mixtures of oligonucleotides was investigated. Hairpin probes were separately heated at 95 °C for 3 min and then allowed cooled to room temperature for 1 h before use. Then, target was incubated with hairpin probes for 24 h at 37 °C to trigger the HCR. Subsequently, TFO was added into the system and the final mixture was further incubated at 25 °C for 3 h. Finally, the circular dichroism spectroscopy was recorded on a spectropolarimeter under the following conditions: room temperature, wavelength from 200 to 320 nm, path length 0.1 cm, response time 1.0 s, bandwidth 1.71 nm. The sample was measured three times.

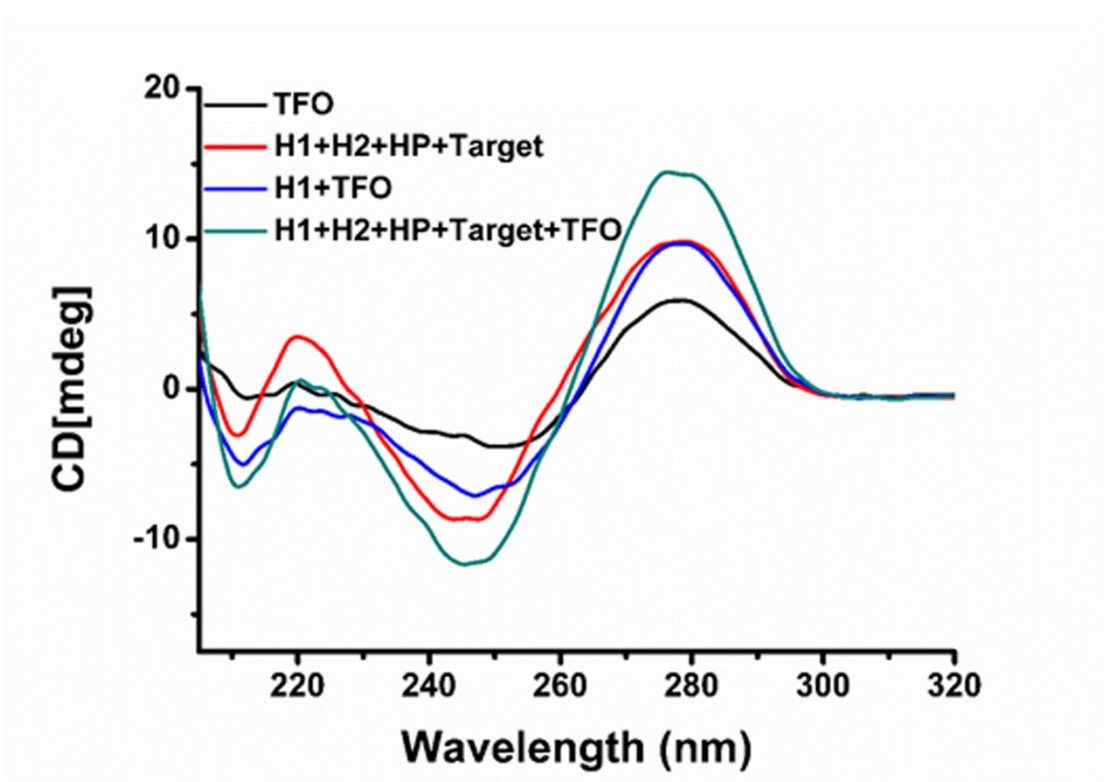

Fig. S2 Circular dichroism (CD) spectroscopy of the oligonucleotides under different conditions.

TFO probes specifically recognized the dsDNA products of HCR

To demonstrate the hypothesis that TFO probes can specifically recognized the dsDNA products of HCR, we investigated the signal change of the sensing system in the presence of various concentration of lambda DNA. The aggregation of AuNPs did not occur and no obvious absorbance change at 522 nm generated. These results suggest that lambda DNA could not be recognized by TFO probes, so we believe that the TFO probes can sequence specifically recognized the dsDNA products of HCR through triplex formation.

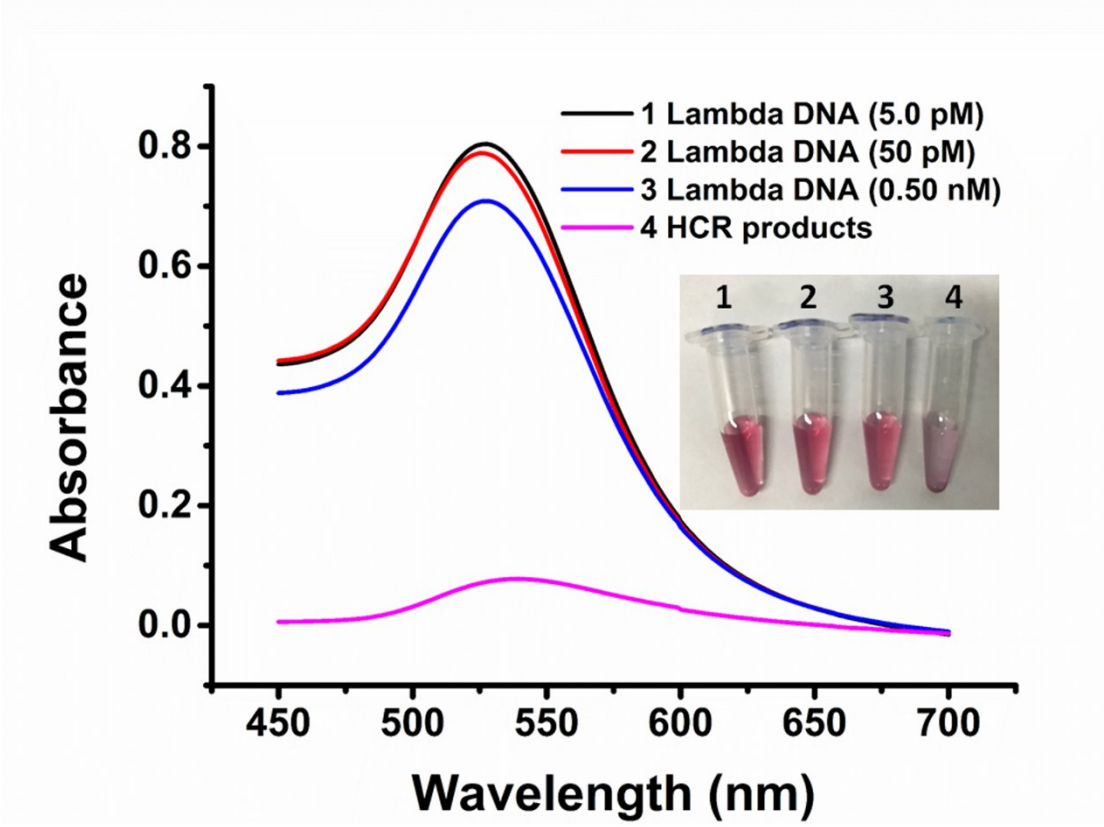

Fig. S3 UV-vis absorption spectra of the colorimetric sensing system in the presence of lambda DNA and HCR products. Inset: photographs of dsDNA-induced color change of the DNA-functionalized AuNPs dispersion.

Optimization of the HCR time and temperature

HCR reaction time and temperature can also affect the performance of the system. As shown in Fig. S4A, the maximized signal was achieved when the HCR time was longer than 3 h. Thus, a HCR reaction time of 3 h was selected for the succeeding experiments. The effect of the HCR reaction temperature o the performance of the sensing system has also been optimized. Fig. S4B shows the temperature could significantly affect the amplification efficiency of HCR, the best efficiency was obtained at 37 °C. Therefore, 37 °C of HCR temperature was selected in future experiments.

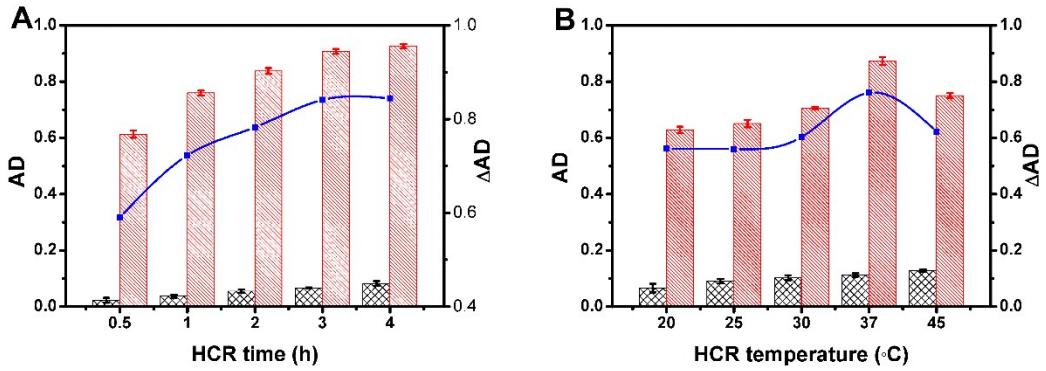

Fig. S4 (A) Effect of the HCR time on the performance of the sensing system. (B) Effect of the HCR temperature on the response of the sensing system. Black columns: control experiments; red columns: with 5.0 nM of target DNA. Blue lines represent the ΔAD at different conditions. The error bars represent the standard deviations of three replicates. The concentrations of HP, H1 and H2 were 5.0 nM, 100 nM and 100 nM, respectively.

Table S1. Comparison of sensors for DNA detection

Detection method	Strategy	Linear range	Detection limit	Ref
Colorimetric	HCR amplification & AuNP & triplex formation	10-500 pM	5 pM	This work
Colorimetric	Enzyme amplification & magnetic separation	25 pM-1 nM	25 pM	3
Colorimetric	HCR amplification & CuNP	0-0.5 nM	0.4 nM	4
Colorimetric	CHA amplification & AuNP	50-300 pM	15 pM	5
SERS	AuNP & graphene	N/A	10 pM	6
SERS	Au particle on wire	10 pM-10 nM	10 pM	7

Table S2. Sequences of oligonucleotides used in this work

Name	Sequence (5'-3')
TFO	**SH-**TTTTTTTTTTTTCTTCTCTTCCTTCTCTTT
T1	CTTCTCTTCCTTCTCTTT
T2	TTTCTCTTCCTTCTCTTC
T3	GAAGAGAAGGAAGAGAAA
Sequences for DNA/RNA sensor	
H1$_{DNA/RNA}$	*ACCTCA*GAAGAGAAGGAAGAGAAAGGTTAATTTCTCTTCCTTCTCTTC
H2$_{DNA/RNA}$	TTTCTCTTCCTTCTCTTC*TGAGGT*GAAGAGAAGGAAGAGAAATTAACC
HP$_{DNA/RNA}$	TTTCTCTTCCTTCTCTTC*TGAGGT*AACTATACAACCTACTACCTCAACCTCAGAAG
Initiator $_{DNA/RNA}$	TTTCTCTTCCTTCTCTTCTGAGGT
Target DNA	TGAGGTAGTAGGTTGTATAGTT
Let-7a	UGAGGUAGUAGGUUGUAUAGUU
Let-7b	UGAGGUAGUAGGUUGUGUGGUU
Let-7c	UGAGGUAGUAGGUUGUAUGGUU
Let-7f	UGAGGUAGUAGAUUGUAUAGUU
Let-7g	UGAGGUAGUAGUUUGUACAGU
Let-7i	UGAGGUAGUAGUUUGUGCUGUU
Sequences for ATP sensor	
H1$_{ATP}$	*CATCTC*GAAGAGAAGGAAGAGAAACCCAGGTTTCTCTTCCTTCTCTTC
H2$_{ATP}$	TTTCTCTTCCTTCTCTTC*GAGATG*GAAGAGAAGGAAGAGAAACCTGGG
HP$_{ATP}$	CCCAGGTTTCTCTTCCTTCTCTTCAAGAGAA**ACCTGGGGGAGTATTGCGGAGGAAGGT**
Initiator $_{ATP}$	CCCAGGTTTCTCTTCCTTCTCTTC
Sequences for PDGF-BB sensor	
H1$_{PDGF-BB}$	*CATCTC*GAAGAGAAGGAAGAGAAAAGCCTGTTTCTCTTCCTTCTCTTC
H2$_{PDGF-BB}$	TTTCTCTTCCTTCTCTTC*GAGATG*GAAGAGAAGGAAGAGAAA*CAGGCT*
HP$_{PDGF-BB}$	GCCGTAGCCTGTTTCTCTTCCTTCTCTTC*TTTTTTTTTT***CAGGCTACGGC** **ACGTAGAGCATCACCATGATCCTG**
Initiator $_{PDGF-BB}$	AGCCTGTTTCTCTTCCTTCTCTTC

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