Electronic Supplementary Information

Self-aggregation of oligonucleotide-functionalized gold nanoparticles and its applications for highly sensitive detection of DNA

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Experimental Section

Materials and Apparatus

DNA oligonucleotides were synthesized by Takara Biotechnology Co. Ltd. (Dalian, China). N. BstNBI nicking enzyme and Vent (exo-) polymerase were purchased from New England Biolabs. Tetrachoroauric acid ($HAuCl_4 \cdot 4H_2O$) was obtained from Sinopharm Group Chemical Reagent Co. (Shanghai, China). NaH_2PO_4, NaOH, and NaCl (Beijing Chemical Reagent Factory, Beijing, China) were of analytical reagent grade and used to prepare the buffer solutions. UV-Visible absorption spectra were measured with a TU1901 Spectrophotometer (Purkinje General Instrument Co., Ltd., Beijing, China). The temperatures were controlled by a Biometra T1 Thermal Cycler (Germany). DSC-T100 (Sony Co., Japan) and D-90 (Olympus, Japan) digital cameras were used to take the photographs. Doubly distilled and sterilized water was used throughout.

Preparation of gold nanoparticles (GNPs)

GNPs were prepared by citrate reduction of $HAuCl_4$ according to Frens[1] and Grabar et al.[2] with slight modification. All glassware was cleaned with chromate washings (cleaning solution), rinsed with water, and oven-dried prior to use. Briefly, after boiling a 100 mL of the 0.01% $HAuCl_4$ solution, 3.5 mL of the 1.0% trisodium citrate solution was quickly added with vigorous stirring. The color of the solution changed to deep red in a few seconds and the reduction of trisodium citrate to $HAuCl_4$ was practically complete after 6-8 min of boiling. The solution was naturally cooled to room temperature and then diluted to 100 mL. The average diameter of the prepared gold nanoparticles was about 15 nm as characterized by transmission electron microscope (TEM, see Fig. S1), and their concentration was estimated by UV/Vis spectroscopy to be about 2.3 nM based on an extinction coefficient of 4.2×10^8 M^{-1} cm^{-1} at $\lambda = 520$ nm.[3]

Functionalization of gold nanoparticles with various DNA densities on the surface

The GNPs were functionalized by 5′-alkanethiol-capped oligonucleotides according to previous literature with minor modifications.[4] Briefly, 0.20, 0.22, 0.26, 0.30, 0.40, 0.50, 1.0, 3.0,

5.0 nmol of the oligonucleotides was added in 1 mL GNP solution and incubated at 45 °C for 24 h, then the solution was adjusted to the pH value and ionic strength of 10 mM sodium phosphate buffer (pH 7.0), and allowed to stand at 45 °C for 12 h. Afterward, the NaCl concentration of the solution was adjusted to 0.05 M by dropwise addition of 2 M NaCl solution. After incubation at 45 °C for another 12 h, this procedure was repeated to adjust the NaCl concentration in the solution to 0.1 M. After subsequent salt aging process for 24 h at 45 °C, the GNPs were isolated by centrifugation for 20 min with 16000 r.p.m. at about 4 ºC and the supernatant was discarded. The red oily precipitate was dispersed by 1 mL of 10 mM phosphate buffer (pH 7.0) containing 0.1 M NaCl. After another centrifugation under the same conditions, the precipitate was re-dispersed with 0.5 mL of the 10 mM phosphate buffer (pH 7.0) containing 0.1 M NaCl and 0.01% Tween-20 to be used as working solution, which contains about 4.6 nM DNA-modified GNPs.

Determination of DNA density on GNP surface

The DNA density on GNP surface was determined according to the literatures.[4] Dithiothreitol (DTT) was added to the DNA-modified GNPs solution to release the DNA from the GNPs into the solution. The final concentrations were 10 mM of DTT and 3.7 nM of DNA-modified GNPs, respectively. After incubation of DTT and DNA-modified GNPs for 18 h at room temperature, the solution containing the released DNA were separated from the GNPs by centrifugation at 12 000 r.p.m. for 5 min, then the released DNA concentration in the supernatant was quantified using OliGreen ssDNA Quantitation Kit and the average DNA density on GNP surface was calculated. The fluorescent intensities were measured with Hitachi F-4500 Spectrofluorimeter (Tokyo, Japan).

Procedure for DNA detection with self-aggregation-based method

The oligonucleotide sequences used in the experiment are listed as follows:

DNA probe: 5'-SH-$(CH_2)_6$- CTGGCGCTTGATGGTA -3'.

Complementary DNA target: 5'- TACCATCAAGCGCCAG -3'.

In a typical experiment for DNA target detection, 30 μL DNA probe-modified GNPs working solution (4.6 nM), 6 μL DNA target in 10 mM phosphate buffer (pH 7.0) and 24 μL 5 M NaCl were added in turn to a 200 μL PCR microtube and the final volume was 60 μL. After incubation of the mixture solution for 10 min at room temperature, the photographs of the solutions were taken by a digital camera, or the absorption spectra were measured by a TU1901 UV-Vis Spectrophotometer.

Specificity evaluation for the self-aggregation-based method

The specificity of the self-aggregation-based method is evaluated by the observation of color and absorption spectrum changes arising from a complementary DNA target, one-base, two-base, and three-base mismatched DNA strands, and a non-complementary DNA strand. The complementary DNA target and the DNA probe immobilized on GNP are the same as mentioned

above. The sequences of one-base mismatched, two-base mismatched, three-base mismatched and non-complementary DNA target are listed as follows (the mismatched bases were underlined):

One-base mismatch DNA target: 5'- TACCATAAAGCGCCAG -3'

Two-base mismatch DNA target: 5'- TACAATCAAGCGACAG -3'

Three-base mismatch DNA target: 5'- TACAATAAAGCGACAG -3'

Non-complementary DNA target: 5'- ATGGTAGTTAGAGGTC -3'

Detection of DNA with self-aggregation-based method coupled with EXPAR

The principle of the EXPAR reaction for DNA amplification was illustrated in Fig. S4. The sequences of DNA target and the amplified templates for EXPAR amplification, and the DNA probes on GNPs are listed as follows:

DNA target: 5'-TACCATCAAGCGCCAG-3', amplified template for DNA target: 5'-CTGGCGCT TGATGGTA**TCCAGACTC**TCTGGCGCTTGATGGTA-P-3', DNA target-specific DNA probe on GNPs: 5'-SH-$(CH_2)_6$- CTGGCGCTTGATGGTA -3'.

EXPAR were carried out in the volume of 10 μL containing 10 mM KCl, 50 mM NaCl, 45 mM Tris-HCl (pH 8.8, 25 °C), 2.0 mM $MgSO_4$, 5 mM $MgCl_2$, 10 mM $(NH_4)_2SO_4$, 0.1% Triton X-100, 0.5 mM DTT, 0.4 units/μL N. BstNBI nicking enzyme, 0.08 units/μL Vent (exo-) polymerase, 400 μM dNTPs, 0.1 μM template and various concentrations of triggering oligonucleotides. Reactions were assembled on ice, initiated by transferring to a preheated thermocycler at 55°C. Finally, the EXPAR products were held at 4°C. Then, 30 μL DNA probe-modified GNPs (26 pmol/cm^2) working solution (4.6 nM), 6 μL EXPAR product, and 24 μL 5 M NaCl were added in turn to a 200 μL PCR microtube. After incubation of the mixture solution for 30 min at room temperature, the photographs of the solutions were taken by a digital camera, or the absorption spectra were measured by the TU1901 UV-Vis spectrophotometer.

Results and Discussion

The characterization of gold nanoparticles with a diameter of 15 nm

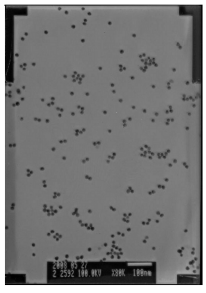

Fig. S1. *The TEM image of the gold nanoparticles.*

Detection of DNA target with non-cross-linking (NCL) aggregation-based method

To compare the sensitivity of the NCL-aggregation-based method and self-aggregation-based method for DNA detection, we detect the same DNA target with NCL-aggregation-based method. The procedure is the same as the self-aggregation-based method except the DNA density on GNP surface and NaCl concentration. As shown in Fig. S2, the lowest concentration of DNA target detectable by the NCL-aggregation-based method is about 200 nM through visualization of GNP color changes, which is consistent with the result previously demonstrated by Maeda et al.[1]

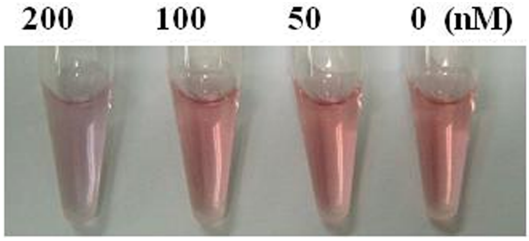

Fig. S2. *Visual detection of DNA target with NCL aggregation-based method. The solutions contain 1.84 nM DNA-GNPs (45 pmol/cm^2) and 1 M NaCl, The hybridization was performed in 10 mM sodium phosphate buffer (pH 7.0) for 10 min at room temperature. The sequences of DNA probe immobilized on GNPs and the complementary DNA target are the same as used in the self-aggregation-based method.*

Evaluation for specificity of the self-aggregation-based method

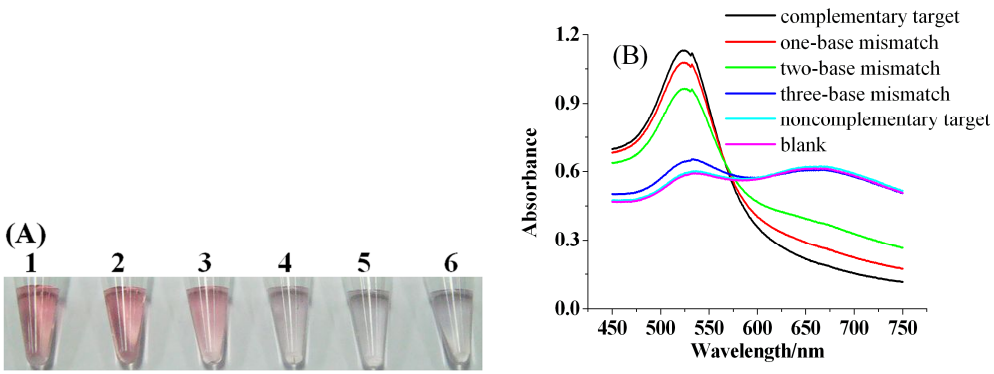

Fig. S3 (A) *Specificity tests for self-aggregation-based method and (B) visible absorption spectra respectively corresponding to the solutions in (A). (1) complementary DNA strands, (2) one-base mismatched DNA strands, (3) two-base mismatched DNA strands, (4) three-base mismatched DNA strands, (5) non-complementary DNA strands, and (6) blank without DNA strands. The solutions contains 2.3 nM DNA-modified GNPs (26 pmol/cm2), 10 nM DNA target, and 2 M NaCl.*

The generality validation for the self-aggregation-based method with different

sequences of DNA probe and DNA target

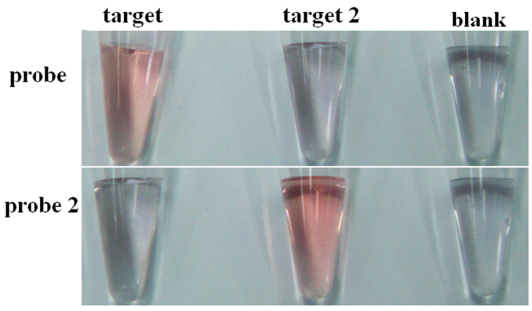

Fig. S4. *Visual detection of DNA target with different sequences with the self-aggregation-based method. The sequences of probe and target are the same as those described in the text for DNA detection and the sequences of probe 2 and target 2 are 5'-SH-$(CH_2)_6$-CTCCACCGCTTCCGAT–3' (26 pmol/cm^2 on the GNP surface) and 5'-ATCGGAAGCGGTGGAG -3', respectively. The reaction condition is the same as described in the Fig. 2 in the text. The concentration of the DNA target and DNA target 2 used in the experiments is 10 nM.*

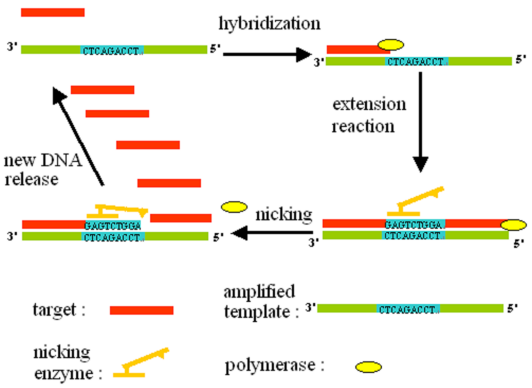

Fig. S5. *Schematic representation of the EXPAR for DNA amplification.*

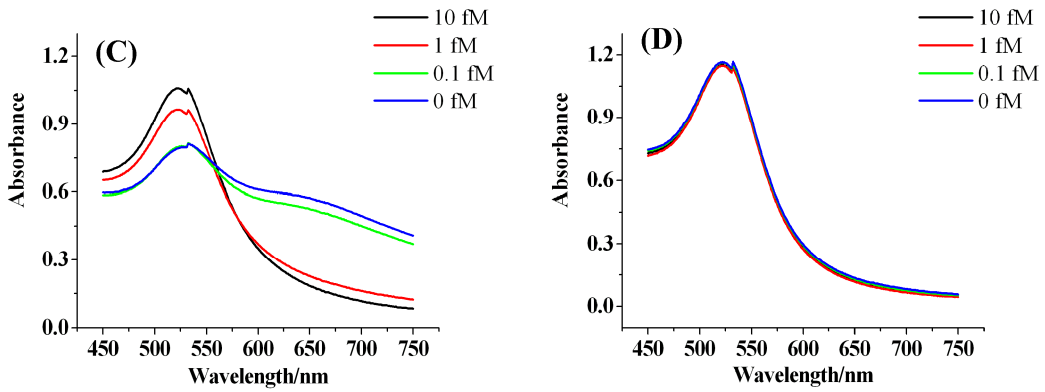

Fig. S6. *Absorption spectra corresponding to the solutions in Fig.4 with the EXPAR reaction time of 6 min 20 sec. (C) and 8 min (D).*

References:

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4 K. Sato, K. Hosokawa, M. Maeda, *J. Am. Chem. Soc.,* 2003, **125**, 8102-8103.