

## Supplementary Information

### Simple, rapid, homogeneous oligonucleotides colorimetric detection based on non-aggregated gold nanoparticles

#### Materials

Oligonucleotides were synthesized by Shanghai Sangon Biological Engineering Technology & Services CO.,Ltd.. All other reagents were of analytical reagent grade and used without further purification. Ultrapure water was used throughout the experiments. The following sequences of DNA oligonucleotides were used in this work:

VV Reporter Probe: 5'-TCTTCCGTTACAAC-(PEG)<sub>6</sub>-Thiol-3'

VV Capture Probe: 5'-NH<sub>2</sub>-CTGATTACTATTGCA-3'

VV Target: 5'-AGTTGTAACGGAAGATGCAATAGTAATCAG-3'

SNP A: 5'-AGTTATAACGGAAGATGCAATAGTAATCAG-3'

SNP T: 5'-AGTTTTAACGGAAGATGCAATAGTAATCAG-3'

SNP C: 5'-AGTTCTAACGGAAGATGCAATAGTAATCAG-3'

VV FAM Reporter Probe: 5'-FAM-TCTTCCGTTACAAC-(PEG)<sub>6</sub>-Thiol-3'

Control DNA: 5'-GGAGTAAATGTTGGAGAACAGTATCAACAA-3'

30 nm Citrate-stabilized Gold nanoparticles (GNPs) were prepared by thermal reduction of HAuCl<sub>4</sub> with sodium citrate. <sup>1</sup> 1.05 μm Dynabeads<sup>®</sup> MyOne™ carboxyl-coated Magnetic Beads (MBs) were purchased from Invitrogen Dynal<sup>®</sup> AS.

#### Equipment

Uv-Vis absorption spectrometer: Shimadzu UV-2550 (Center of Analysis and Test of Wuhan University)

Transmission Electron Microscope: JEM-2100 (HR)

Acceleration Voltage : 200KV Emitter: LaB6 JEOL Ltd.

(Center of Analysis and Test of Wuhan University)

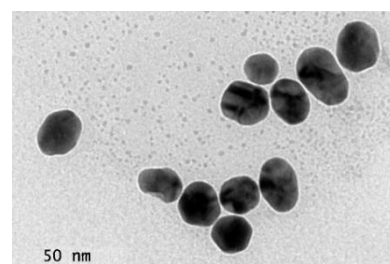


Fig S1. TEM image of 30nm Gold nanoparticles modified with reporter probes

#### Preparation of Magnetic Beads Modified with Capture Probe

The carboxylated magnetic beads were conjugated with the capture DNA using the protocol suggested by the manufacturer. Before immobilization, 2.5 mL of carboxylated magnetic beads were washed twice with 2.5 mL of MES buffer (100 mM, pH 4.8) and the beads were resuspended in 250 μL MES buffer (100 mM, pH 4.8). Next, mixture of 36.2 nmol NH<sub>2</sub>-modified capture DNA and 36.2 μmol EDC in 100 μL MES buffer was added to the washed magnetic beads and incubated at room temperature with gentle shaking overnight. Finally, the coated magnetic beads were incubated with 50 mM Tris buffer (pH 7.4) for 15 min at room temperature with gentle shaking to quench the unreacted activated carboxylic acid groups. The coated magnetic beads were washed three times with 2.5 mL Tris buffer and then resuspended in hybridization buffer (10 mM phosphate sodium buffer solution, pH 7.4, 300 mM NaCl, 0.05% Tween 20) and stored at 4 °C.

#### Preparation of GNPs Modified with Reporter Probe

#### *Gold nanoparticles modification (0.1M salt aged)*

15 nmol PEG-thiol-functionalized reporter probe was added to 5 ml of gold nanoparticles (30 nm) suspended in PBS (pH 7.4, 10 mM phosphate). After 24 hrs, aqueous 2M NaCl was added to the solution to bring the total NaCl concentration of the probe solution to 0.05 M, after standing for 8 hrs, the NaCl concentration of the probe solution increased to 0.1 M. Upon aging in 0.1 M salt for an additional 40 hrs, the nanoparticles were isolated by centrifugation 6500 rpm for 15 min with glass tube and washed three times with PBS-T (pH 7.4, 10 mM phosphate, 0.05% Tween-20).

#### *Gold nanoparticles modification (0.7M salt aged)*

These particles were modified in a manner analogous to that described above. After reach the 0.1M NaCl, the NaCl concentration increased 0.05M for every 8 hrs standing. The GNPs aged in 0.7M salt for 40 hrs before centrifugation.

### **Detection of Sequence-Specific DNA**

In a typical non-aggregated GNPs based DNA detection experiment, 1  $\mu$ L sample solution containing target DNA in the single-stranded form was added to 14  $\mu$ L MBs probes (10 mg mL<sup>-1</sup>, PBS-T buffer) solution to initiate the assay. The system was allowed to gentle vortex at room temperature for 30 min. The GNPs probes (65  $\mu$ L, PBS-T buffer) were then added to the solution and allowed to hybridize with gentle vortex for 30 min. The total volume of system was 80  $\mu$ L. After hybridization, the MBs with target-linked GNPs along with unreacted MBs were easily pulled to the wall of the tube in just about one minute by applying external magnetic field. After the separation, supernatant was tested by UV-2550 with a 50  $\mu$ L Quartz Micro-cuvette. The whole process was carried out at room temperature  $23 \pm 2^\circ\text{C}$ .

### **Quantification of PEG-thiol Oligonucleotides Loaded on GNPs**

We detected the DNA quantification on 30nm gold nanoparticles after 0.1M and 0.7M salt age process by literature method.<sup>2</sup> VV FAM Reporter Probe was used to produce a fluorescent signal. The amount of DNA on single GNP can be adjusted by the salt concentration of age process, the higher salt concentration the more probes loaded on single GNP.

Salt concentration of aging process	0.1M	0.7M
Amount of DNA on single GNP	240	700

### **Analysis on the Relationship of Amount of DNA Probe Loading on Single GNP to Sensitivity**

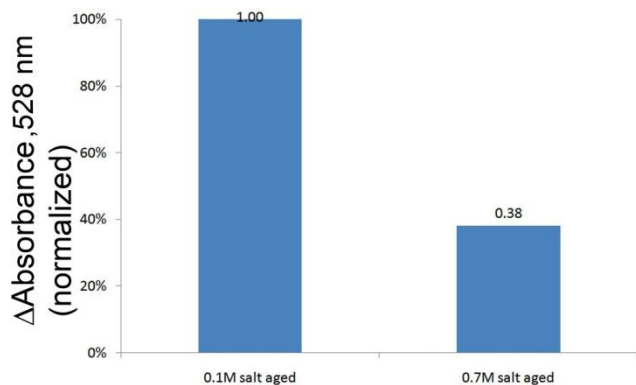


Fig S2. Normalized absorbance change when 80fmol target was added using 0.1M salt aged GNPs(left) and 0.7M salt aged GNPs(right).

Assuming more surface loading amount of target oligonucleotide receptors on 30 nm GNPs, the assay sensitivity will drop directly with the increase of probe surface loading amount. This is due to the fact that the more the reporter oligonucleotides loaded on GNPs, the more oligonucleotide targets will be attached to each particle. Therefore, when one compares 0.1 M salt aged and 0.7 M salt aged 30 nm particles, the factor in the change of sensitivity from 0.1 M salt aged to 0.7 M salt aged GNPs should be approximately  $240 / 700$  or 0.34. The experimentally determined value was 0.38 ( $\Delta\text{Abs}_{0.7\text{ M}} / \Delta\text{Abs}_{0.1\text{ M}}$ ) based on the same condition (80 fmol target DNA, pH 7.4, 10 mM phosphate, 300 mM NaCl, 0.05 % Tween-20). One could further increase the sensitivity by decreasing the absolute surface coverage of oligonucleotides.

### The Stability of Non-aggregated GNPs

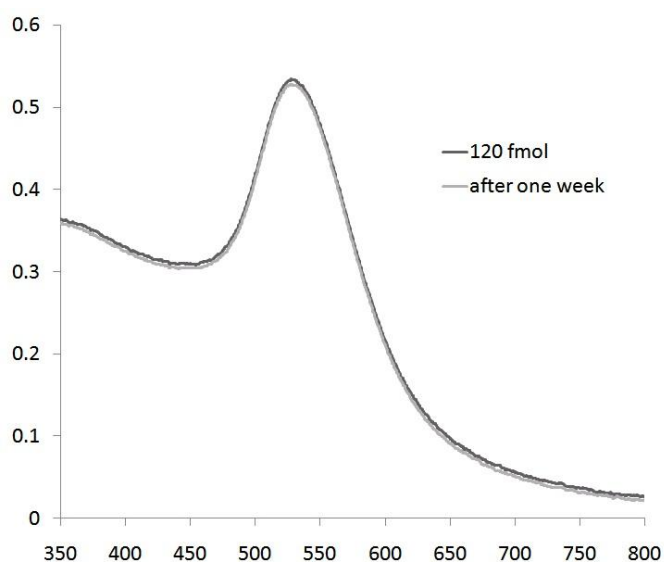


Fig S3. UV-Vis spectra of fresh GNPs(black) and GNPs stored for one week(grey).

The non-aggregated GNPs are stable in high ion strength. To test the stability of non-aggregated GNPs, the GNPs supernatant after hybridized with 120 fmol Target has been chosen. No significantly difference can be observed after one week store. The experimental result proves that the non-aggregated GNPs based colorimetry for oligonucleotides detection is a stable method.

### Detect Target DNA from Mixture

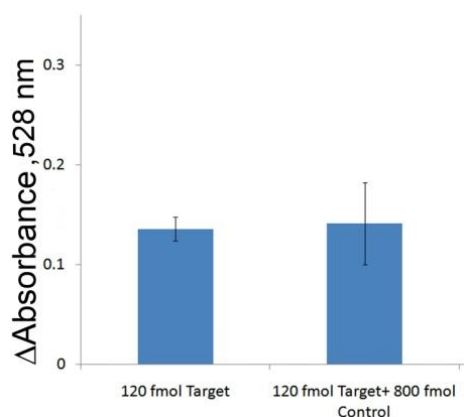


Fig S4. Absorbance change of 120 fmol target DNA(left) and mixture of 120 fmol target and 800 fmol control DNA (right).

To test the selectivity of our assay, 800 fmol control DNA mix with 120 fmol target DNA have been added to the detection systems. The results show no significantly difference which prove that our assay is high selective.

### The Relationship of Amount of DNA-GNP Probe to Sensitivity

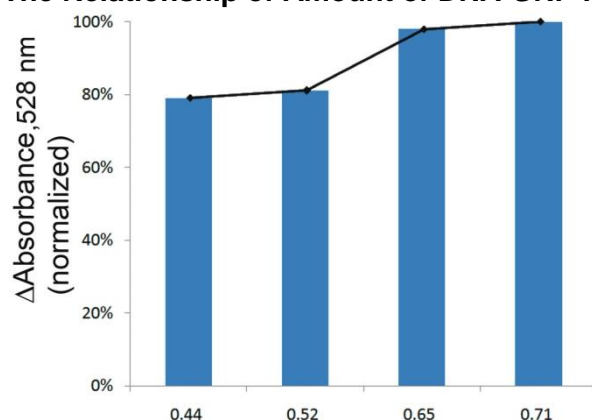


Fig S5. Normalized absorbance changes when the initial concentration of DNA-GNP changed.

When the initial concentration of DNA-GNP changed, the sensitivity only have little changes compare with the change caused by different amount of DNA loading on single GNP. This change may be due to the different number ratio of the GNPs and MBs. The sensitivity could be further improved by changing the size of particles and number ratio of GNPs and MBs. (80 fmol target DNA, pH 7.4, 10 mM phosphate, 300 mM NaCl , 0.05 % Tween-20)

### Reference

- (1)G. Frens, *Nature*, 1973, **241**, 20-22
- (2)S. J. Hurst, A. K. R. Lytton-Jean and C. A. Mirkin, *Anal Chem*, 2006, **78**, 8313-8318