Electronic Supporting Information

for

# Colorimetric detection of single-nucleotide polymorphisms with a real-time PCR-like sensitivity

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**Materials and Apparatus.** Amine-terminated capture probes (CPs) and all other oligonucleotides were obtained from Proligo Singapore Pte Ltd (Singapore). Sequence information of the oligonucleotides is given in Table S1. The engagement of a pair of partial probes is to produce secondary targets necessary for the configuration of LCR.[1] The Ampligase® thermal stable ligase kit was from Epicentre Biotechnologies (Madison, USA). Zonyl FSN-100 and 1,4-phenylenediisothiocyanate (PDITC, 98%) were purchased from Sigma-Aldrich. AuNPs (20-nm) were obtained from Ted Pella (Redding, CA). All other reagents of certified analytical grade were used as received. Nuclease-free ultrapure water was used for all solution preparations.

Most of the UV-Vis absorbance experiments were conducted on a multi-detection microplate reader (Tecan, Switzerland). The measurements were carried out in 384-well microplates. In some experiments, the UV-Vis absorbance spectra were acquired on a G1103A UV-Vis spectrophotometer equipped with a Peltier temperature controller from Agilent Technologies (Santa Clara, USA). A GeneAmp 9600 Thermal Cycler from PerkinElmer (Waltham, MA, USA) was used for PCR amplification of genomic DNA.

**Synthesis of disulfide-based polyamidoamines (mixed oligomers):** 10 ml of tris(2-aminoethyl) amine, 60 ml of methanol and 17 ml of epichlorohydrin were placed in a flask under nitrogen. The mixture was left to stir in an ice bath for 24 h. Subsequently, 250 ml of pentaethylenehexamine and 100 ml dimethyl sulfoxide were then added to the flask. The mixture was left to stir at 90°C for 24 h. The reaction mixture was then dialyzed in water using dialysis tubing with a molecular weight cut-off of 500 Da (Spectrum Laboratories, USA) for 24 h, followed by freeze drying. Next, 10 g of the freeze-dried intermediate (amine monomer) was added to a flask containing 150 ml of methanol and 1.5 ml of methyl-3-mercaptopropionate. The mixture was then left to stir at 60°C for 24 h. The excess methanol was removed under vacuum and the mixture was precipitated in THF and vacuum-dried to give the disulfide-based polyamidoamines (mixed oligomers) (yield: 70%). The $M_w$ of the disulfide-based polyamidoamines was determined to be 2.7 kDa with a polydispersity index of 1.3.

**Functionalization of AuNPs.** To minimize the nonspecific adsorption of the CP strands and to increase the stability of the immobilized CPs, the AuNPs were first functionalized with the disulfide-based polyamidoamines containing multiple disulfide groups for the formation of robust coatings on the AuNPs, and primary amine groups for further conjugation.[2] Briefly, before introducing to the disulfide-based polyamidoamines, the AuNPs were first coated with Zonyl FSN-100 by adding ~0.05%

Zonyl FSN-100 to the AuNP solution.[3] The disulfide-based polyamidoamines were then mixed with the Zonyl FSN-100 coated AuNPs at concentrations of 1% of the disulfide-based polyamidoamines and 2.0 nM of the AuNPs in pH 7.4 phosphate buffered saline (PBS). The mixture was allowed to incubate at room temperature for 2 h. The functionalized AuNPs were separated and purified from the reaction mixture by three cycles of 15-min centrifugation at 20000 x g and redispersion in PBS. Now, the AuNPs were ready for the amine-terminated CP grafting. 50 mg PDITC was added to a mixture solvent of 1.0 ml dimethylformamide and 1.0 ml pyridine. Next, the amine-terminated CPs were allowed to react with the solution to activate the amines for 4-6 h, followed by a butanol/water extraction. The aqueous solution containing the activated CPs was lyophilized. The activated CPs were then dissolved in borate buffer and reacted with the coated AuNPs in a pH 9.2 0.10 M phosphate buffer overnight. The functionalized AuNPs were separated and purified from the reaction mixture by three cycles of 15-min centrifugation at 20000 x g and redispersion in the pH 7.4 PBS. The concentration of the CP-coated AuNPs was estimated by measuring the localized surface plasmon resonance (LSPR) absorbance of the AuNPs at 520 nm using the molar absorptivity value of $8.0 \times 10^8$ $Lmol^{-1}cm^{-1}$.[4] Two types of the CP-coated AuNPs were prepared so that the target gene could act as a template to perfectly align a pair of the AuNP probes along the target and the two termini of the CPs meet at the mutation site.

**Genomic DNA sample preparation.** Tissue specimens were obtained with informed consent from Singapore Tissue Repository. Genomic DNAs in 5-10 mg of the tissue specimens were extracted using the QIAamp tissue extraction kit (Qiagen, Germany), following the recommended protocol. Genomic DNAs from cultured cell line SW480 (KRAS codon 12 homozygous GTT) and K562 (wild-type) were extracted by the Nucleospin DNA extraction kit (Invitrogen, Germany). The entire coding region of exon 2 of KRAS (224 bp) was amplified using the following primer pair: 5'-GTG TGA CAT GTT CTA ATA TAG TCA-3' and 5'-CTG TAT CAA AGA ATG GTC CTG CAC-3'. PCR amplification was performed in 100 μl mixture containing 100 ng of genomic DNA under the following conditions: an initial melting of 10 min at 95°C; 35 cycles of 30 s at 90°C, 30 s at 60°C and 30 s at 72°C; and a final extension of 5 min at 72°C. Amplified products were purified using spin columns (Qiagen, Germany) and confirmed by DNA sequencing.

**Ligation chain reaction (LCR) amplification.** 100 μL of reaction solution containing Ampligase reaction buffer, 100 U Ampligase, 20 nM of the two types of the CP-coated AuNPs, 0.5 μM of each of the partial probe A and B, and the KRAS wild-type target was vortexed thoroughly and divided into five equal aliquots. The five aliquots were simultaneously subjected to thermal cycling. After a thorough optimization process, the LCR protocol was established as follows: The thermal cycle was composed of 2 min ligation at 45°C and 1 min denaturation at 85°C. After each thermal cycle, the absorbance ratio of $A_{640nm}/A_{520nm}$ was measured and subtracted from that of the control. The cycle threshold ($C_T$) with an absorbance ratio of $10x\sigma$ ($\sigma$: the standard deviation of 6 control experiments) was used to construct the calibration curve. Direct visual inspection was done when only qualitative genotyping is necessary.

The color change upon hybridization and ligation and the corresponding UV-Vis absorption spectra of the CP-coated AuNPs and the ligated AuNP aggregates templated by the target are compiled in Fig. S1. It was found that the characteristic absorption spectrum, due to localized surface plasmon resonance (LSPR) of the AuNPs, was practically identical for both the uncoated and the CP-coated AuNPs in

PBS. A ~120-nm red-shift of the LSPR absorption peak was observed in the presence of the target, apparently due to the aggregation of the AuNPs.[5]

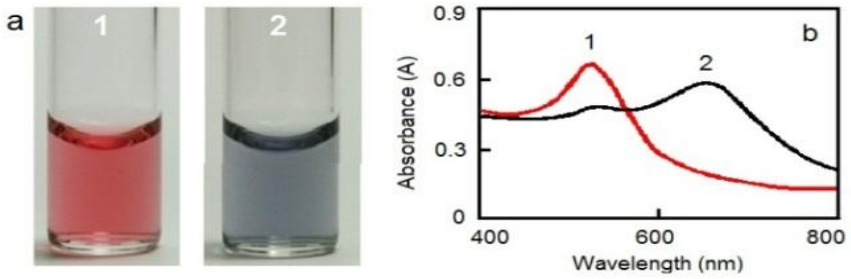


**Fig. S1**. (a) Color change and (b) UV-Vis spectra of 4.0 nM of 1:1 mixture the two types of the capture probe-coated AuNPs (1) before and (2) after the addition of 100 nM target and Ampligase.

**Table S1.** Oligonucleotides used in the assay.

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| **Common capture probe** | 5'-$NH_2$-TTTTCTTGCCTACGCCAC-OH-3' |
| **Capture probe A (CP-A)** | 5'-$PO_4^-$-**A**AGCTCCAACTACCACTTT-$NH_2$-3' |
| **Capture probe T (CP-T)** | 5'-$PO_4^-$-**T**AGCTCCAACTACCAC-TTTN$H_2$-3' |
| **Capture probe C (CP-C)** | 5'-$PO_4^-$-**C**AGCTCCAACTACCACTTT-$NH_2$-3' |
| **Capture probe G (CP-G)** | 5'-$PO_4^-$-**G**AGCTCCAACTACCACTTT-$NH_2$-3' |
| **Common partial probe A** | 5'-$PO_4^-$-GTGGCGTAGGCAAGA-3' |
| **Partial probe B for WT** | 5'-TGGTAGTTGGAGCT**G**-OH-3' |
| **Partial probe B for MT-A** | 5'-TGGTAGTTGGAGCT**A**-OH-3' |
| **Partial probe B for MT-T** | 5'-TGGTAGTTGGAGCT**T**-OH-3' |
| **Partial probe B for WT-C** | 5'-TGGTAGTTGGAGCT**C**-OH-3'' |
| **KRAS wild-type target (WT)** | AAACTTGTGGTAGTTGGAGCT**G**GTGGCGTAGGCAAGAGTGCCTTG |
| **KRAS mutant target A (MT-A)** | AAACTTGTGGTAGTTGGAGCT**A**GTGGCGTAGGCAAGAGTGCCTTG |
| **KRAS mutant target T (MT-T)** | AAACTTGTGGTAGTTGGAGCT**T**GTGGCGTAGGCAAGAGTGCCTTG |
| **KRAS mutant target C (MT-C)** | AAACTTGTGGTAGTTGGAGCT**C**GTGGCGTAGGCAAGAGTGCCTTG |

## References

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