

Supporting Information:

Colorimetric Detection of *c-Kit* Mutation Using Electrostatic Attraction Induced Aggregation of Peptide Nucleic Acid Modified Gold Nanoparticles

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Experimental Details

Oligonucleotide targets and PNA probes: Three different types of purified target oligonucleotides derived from exon 11 of *c-Kit* gene were synthesized by Bioneer Inc. (Daejeon, Korea), and purified by the polyacrylamide gel electrophoresis method: i) wild type; ii) point mutation in codon 559; iii) 6-bp deletion mutation in codon 557/558. Each type of target oligonucleotides was synthesized in three different lengths (12-, 21-, and 42-mer) to study effect of target DNA length. PNA probe that is complementary to the wild type target sequence was designed to contain N-terminal thiol and six carbon spacers between the thiol and the PNA sequences, and purchased from Panagene (Daejeon, Korea).

Preparation of AuNPs and surface modification: Citrate reduction method was used to synthesize the negatively charged AuNPs. We dissolved 10 mg of HAuCl₄·3H₂O (Sigma-Aldrich) in 100 mL of deionized water and heated the solution. After boiling, 1.5 mL of 1 wt.% tri-sodium citrate solution was added to aurate solution and the mixture was further

heated until the color changed to red. Average hydrodynamic diameter and zeta potential of the negatively charged AuNPs were estimated to be ~18 nm and -24 mV, respectively.

Positively charged AuNPs were prepared by Niidome method.²¹ We dissolved 55 mg of HAuCl₄·3H₂O in 100 mL of deionized water, and added 24 mg of cysteamine hydrochloride to the aurate solution. The mixture was stirred until color changed to light brown. After stirring for 30 min, 2.5 mL of 1 mM NaBH₄ solution was added dropwise for ~5 min. After reacting for 12 h, the solution was dialyzed for 1 h in deionized water with cellulose-ester membrane (molecular weight cut-off: 8,000~10,000) to remove residual chemicals. Average hydrodynamic diameter and zeta potential of the positively charged AuNPs were ~30 nm and +28 mV, respectively.

For surface modification, 50 µL of 0.1 mM PNA probe solution was added to 1 mL of the negatively charged AuNP suspension. After reacting for 1 h at room temperature, the solution was dialyzed for 30 min to remove residual PNA probes and chemicals, and redispersed by sonication.

Colorimetric assay procedure: For hybridization between target oligonucleotide and PNA on the AuNP surface, 1 µL of 40 µM target oligonucleotide solution was mixed with 40 µL of the PNA-modified AuNP suspensions. After hybridization for 5 min, the suspension was centrifuged at 10,000 rpm for 5 min to remove residual target oligonucleotides, and redispersed in 40 µL of deionized water by sonication. We then added 40 µL of the DNA/PNA-modified AuNP suspension to the positively charged AuNP suspension which was prepared by diluting 60 µL of the positively charged AuNP suspension with 240 µL of deionized water. Total target DNA concentration was 0.1 µM.

Characterization: UV–vis absorption spectroscopy was used to observe the spectral changes of the AuNP suspensions with Shimadzu 1650 PC spectrophotometer. Average hydrodynamic diameter and zeta potential of AuNPs were estimated by the Malvern Nano–ZS instrument (Malvern, UK). TEM (JEOL JEM 2100) was used to observe the morphology of the AuNPs.

Table S1. Sequences of probe PNA and target oligonucleotides

Type		Sequences ^a
PNA probe		N ³ -SH-C6-AAC AAC CTT CCA CTG TAC TTC-C'
12-mer targets	deletion mutation	5'- GTA CAG GTT GTT-3'
	point mutation	5'- TGG AAG GMT GTT-3'
	wild type	5'- TGG AAG GTT GTT-3'
21-mer targets	deletion mutation	5'-ATG TAT GAA GTA CAG GTT GTT-3'
	point mutation	5'-GAA GTA CAG TGG AAG GMT GTT-3'
	wild type	5'-GAA GTA CAG TGG AAG GTT GTT-3'
42-mer targets	deletion mutation	5'-TAC AAA TAT TTA CAG AAA CCC ATG TAT GAA GTA CAG GTT GTT-3'
	point mutation	5'-TAT TTA CAG AAA CCC ATG TAT GAA GTA CAG TGG AAG GMT GTT-3'
	wild type	5'- TAT TTA CAG AAA CCC ATG TAT GAA GTA CAG TGG AAG GTT GTT-3'

^a 'M' stands for (A or C)

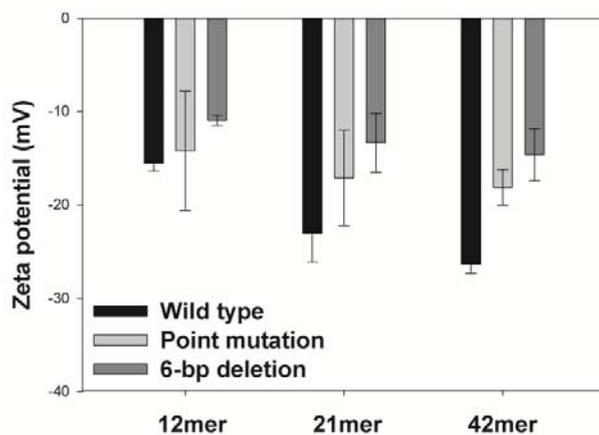


Fig. S1. Zeta potential of AuNP suspensions after hybridization with three types of target oligonucleotides with different length

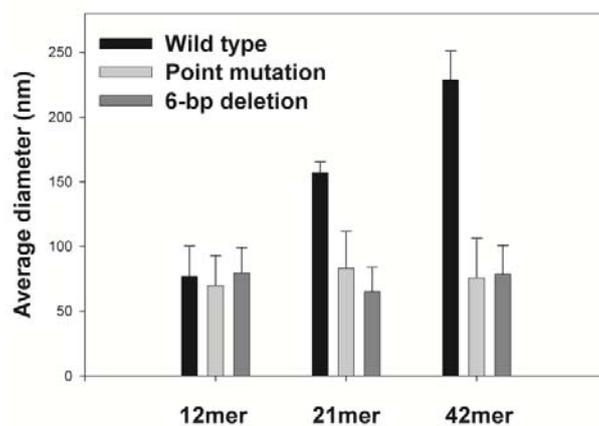


Fig. S2. Average diameters of AuNP suspensions for three types of target oligonucleotides after addition of the positively charged AuNPs

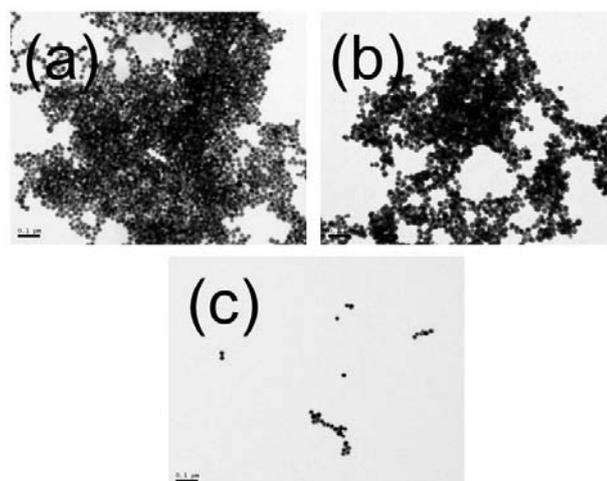


Fig. S3. TEM images of AuNP suspensions after addition of the DNA/PNA-modified AuNPs for wild type target oligonucleotides with different length: (A) 42-mer; (B) 21-mer; (C) 12-mer.