Supplementary Material (ESI) for Chemical Communications

Label-Free and Naked Eye Detection of PNA/DNA Hybridization Using Enhancement of Gold Nanoparticles

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MATERIALS AND METHODS

Preparation of oligonucleotide

All DNAs and PNAs were synthesized by Bioneer Co. and Panagene Co. in Korea, respectively. Table S1 shows the sequences of the PNA probes and the complementary DNA targets. Probe A is the PNA probe, which captures the synthetic 18-mer target DNA (target A) and the Cy3-labeled synthetic 18-mer target DNA (target A′) derived from the H5-type avian influenza virus. Probe B is the PNA probe, which is complementary to the randomly sequenced 50-mer DNA (target B).

Surface modification of slide glass

The slide glass was treated with piranha solution (H_2O_2:H_2SO_4 = 1:3) and oxygen plasma to make a hydroxyl-terminated surface. The glass was reacted with 2% aminopropyltriethoxysilane (APTES, Sigma) in ethanol for 2 hrs, washed with ethanol and baked at 120°C for one hour. The amine-terminated glass chip was further modified by overnight immersion in a solution of 1 M succinic anhydride (Sigma) in dimethylformamide (DMF) solvent and subsequently washed with DMF and deionized water under ultrasonication for 5 min. The carboxylic acid-terminated surface of the glass was then created. After the carboxylic acid-modified glass slides were treated with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and N-hydroxysulfosuccinimide (sulfo-NHS) for 15 min, a

50 μM amine-terminated PNA probe in 1× spotting buffer (Panagene, Korea) was incubated for 1 hr by the microarray technique at room temperature. To block the unreacted glass surface, 1 mM amine-modified polyethylene glycol (PEG, M.W. = 2,000, Sunbio, Korea) was treated for 2 hrs at room temperature and washed with water.

Preparation of nanoparticles

We prepared six sizes of gold nanoparticles (1.4, 4, 15, 20, 30, and 40 nm), five commercially available colloid solutions and one synthetic colloid solution. Positively charged 1.4 nm gold nanoparticles [10] were purchased from Nanoprobes (USA). Gold nanoparticles with diameters of 15, 20, 30 and 40 nm were purchased from BBI International (USA). Gold nanoparticles 4 nm in size were synthesized in the laboratory with chemicals purchased from Sigma. A solution prepared by dissolving 1.97 mg $HAuCl_4$ in 0.38 mM citric acid solution (20 mL) was reduced by a freshly prepared 0.1 M sodium borohydride ($NaBH_4$, 0.6 mL) solution at room temperature. Then, 0.2 M cetyltrimethylammonium bromide (CTAB) was added to each gold nanoparticle solution at the same volume ratio to make the surface of the particles positively charged. Finally, the gold nanoparticle solution in CTAB solution was mixed with 1× phosphate buffered saline with Tween (PBST containing 0.1% Tween, pH 7.4) at a volume ratio of 1:1 before it was introduced to the DNA hybridized substrate. The synthesized nanoparticles were characterized by transmission electron microscopy (TEM) and a particle analyzer. TEM images were obtained from a JEOL, JEM-2100F electron microscope (Japan) operated at 200 kV. Zeta-potential analysis was performed using ELS-Z, Otsuka Electronics (Japan). The values were obtained by averaging three individual measurements.

DNA detection

The PNA-modified glass chips were hybridized in solutions containing different concentrations of target DNA in 1× PBST at 37°C for 1 hour with slight shaking. After the glass was intensively washed

with PBST and PBS, a solution containing the positively charged gold nanoparticles was dropped onto the chip surface and incubated for 30 min at room temperature. After intensive washing with PBST and PBS, the surface was reacted with gold enhancement solution (Nanoprobes, USA) for 1 min.

Optical images were obtained using an optical flat-scanner (Epson Perfection V30) with a resolution of 1,200 dpi (dots per inch), and the data was analyzed with an 8-bit grayscale histogram in Photoshop (Adobe Systems). The fluorescent images were obtained by a fluorescent scanner (Genefix 4200A, Molecular Devices), with settings of 350 PMT and 70% power. The targeted surface of the substrate was assessed using a scanning electron microscope (Sirion, FEI).

Measurement of melting temperature

The melting temperature of PNA/DNA in PBST buffer was measured by Beckman Coulter UV/Vis optical absorption spectra at 260 nm with peltier temperature controller. The data were recorded at 1℃ intervals with 1 min heating time and a hold time of 2 min at each temperature, while the sample (1 μM) was heated from 20℃ – 85℃. The melting temperature of 62℃ for the PNA/DNA duplex (target A) was obtained.

Supplementary Information

A TEM image showed that the prepared gold nanoparticles were 4 nm in diameter and spherical in shape (Fig. S1). The surface charge of the gold nanoparticles in various solutions was measured by using a zeta potential analyzer. The zeta potential value was changed by altering the solutions (see Table S2). The bare gold nanoparticles dissolved in citric acid solution were negatively charged (-16.71 mV). However, the zeta potential value became positively charged (19.95 mV) with the addition of CTAB into the gold nanoparticle solution. Additionally, it was found that the zeta potential value was slightly decreased (15.46 mV) when PBST buffer was added into the CTAB-coated gold nanoparticle solution. The zeta potential values of commercial gold nanoparticle (CTAB coated) in PBST solution are shown in Table S3. The surfaces of all the gold nanoparticles tested were found to be positively charged.

Table S1. Oligonucleotide sequences for probes and targets used in this research.

Name	DNA sequence (5′ – 3′)
Probe A* (probe PNA)	NH_2-O-AAT GGT TTA TTC TGC TCA (18 mer)
Target A (target DNA)	TGA GCA GAA TAA ACC ATT (18 mer)
Target A′ (Cy3-target DNA)	Cy3-TGA GCA GAA TAA ACC ATT (18 mer)
Probe B (probe PNA)	NH_2-O-GAC ATC AAG CAG CCA TC (17 mer)
Target B (long target DNA)	AGC TTC CTC ATT GAT GGT CTC TTT TAA CAT
	TTG GAT GGC TGC TTG ATG TC (50 mer)
Single-base mismatched DNA	TGA GCA **T**AA TAA ACC ATT
Target DNA mimic*	TAG TGG AGA AGG CCA GTC CAG CCA ATG ACC TCT GTT ACC CAG GGG ATT TCA ACG ACT ATG AAG AAC TGA AAC ACC TAT **TGA GCA GAA TAA ACC ATT**

(* the sequence was originated from influenza A virus (H5N1) segment 4 hemagglutinin (HA) gene.)

Solution for gold nanoparticles dispersion	Zeta potential (mV)
0.38 mM citric acid	-16.71 ± 2.47
0.1 M CTAB	19.95± 2.89
0.05 M CTAB+PBST	15.46 ± 2.92

Table S2. Zeta potential values of 4 nm gold nanoparticles dispersed in various solutions.

Particle size	Zeta potential (mV)
1.4 nm	Not measured*
15 nm	15.90 ± 0.87
20 nm	14.46 ± 2.16
30 nm	13.95 ± 0.40
40 nm	11.55 ± 2.05

Table S3. Zeta potential values of the commercial gold nanoparticles in PBST solution.

(*: According to the manufacturer, the reliable value of zeta potential cannot be obtained with gold nanoparticles of smaller than the about 3 nm.)

Solution without gold nanoparticles	Zeta potential (mV)
0.2 M CTAB	> 35
0.1 M CTAB + PBST	0.55 ± 1.42

Table S4. Zeta potential values of the each solution without gold nanoparticles.

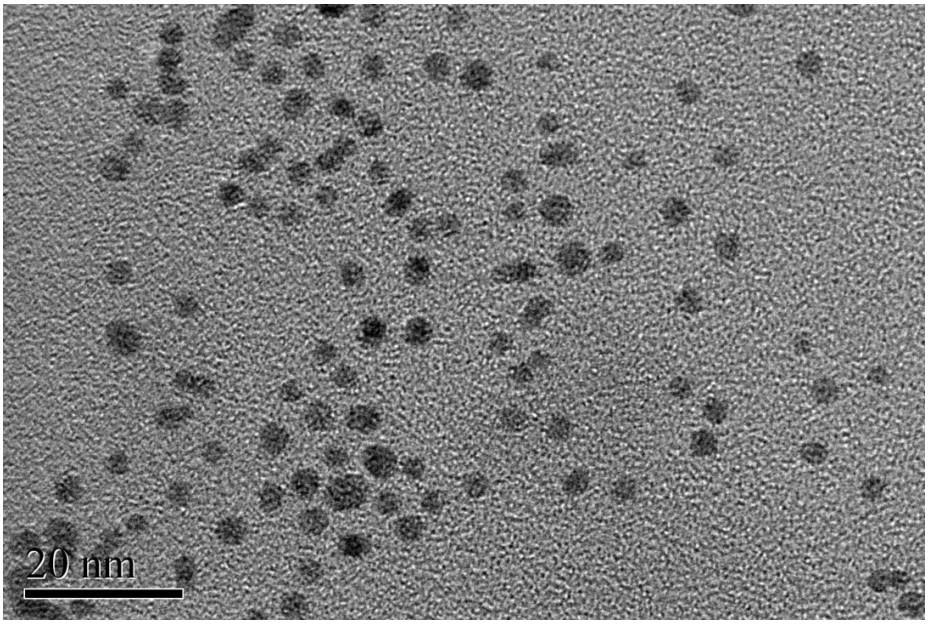

Figure S1. TEM image of the synthesized gold nanoparticles

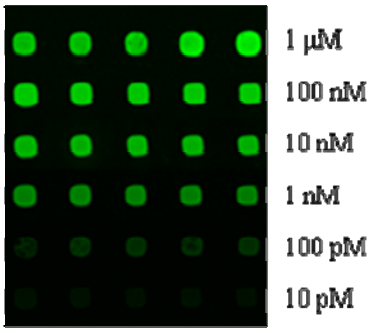

Fig. S2. Fluorescent images of DNA chips with different concentrations of the Cy3-labeled target A′. The concentrations of target DNA, from top to bottom, were 1000, 100, 10, 1, 0.1, and 0.01 nM. The fluorescence detection limit was 10 pM.

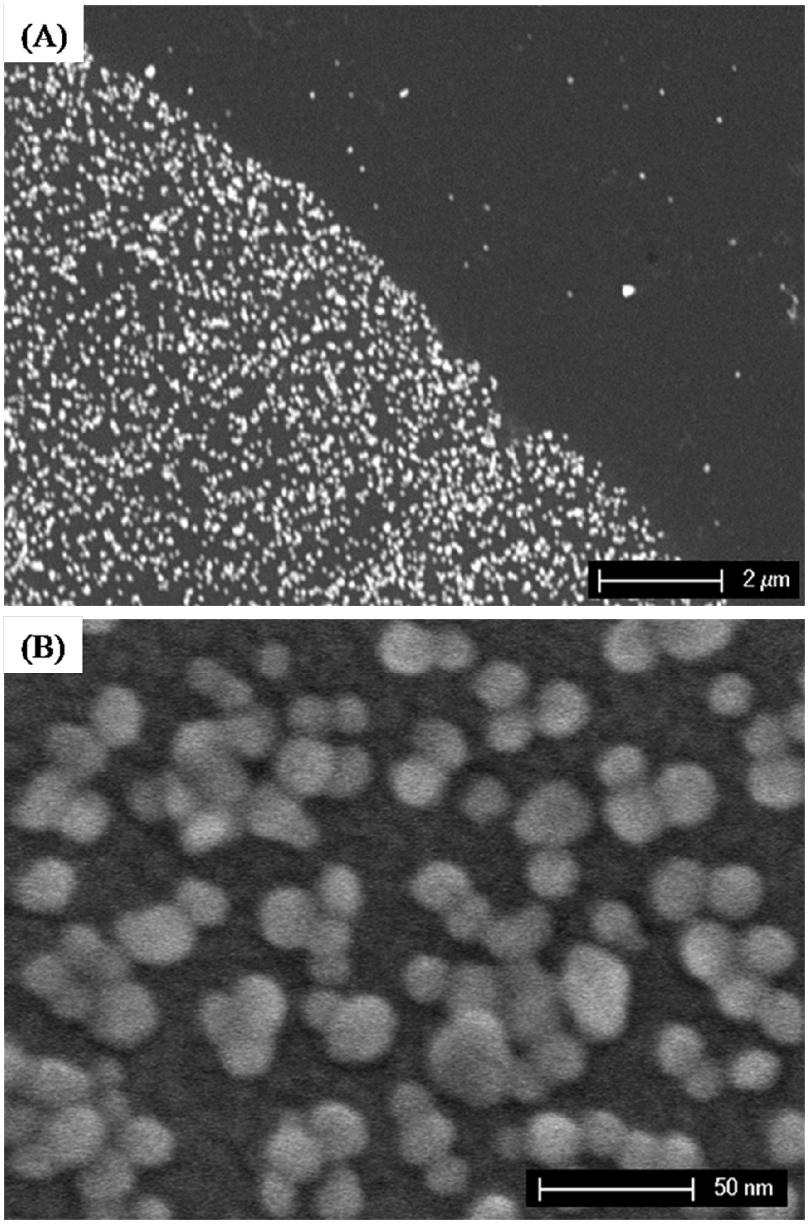

Figure S3. SEM image of the targeted spot (10 nM) after gold enhancement process. (A) The border between the inside and outside of the spot was clearly distinguished, indicating that the positively charged gold nanoparticles specifically bound to the target-hybridized PNA probes. (B) The magnified image shows that the size of the gold nanoparticles was found to be *ca.* 10 nm to 30 nm after enhancement process.

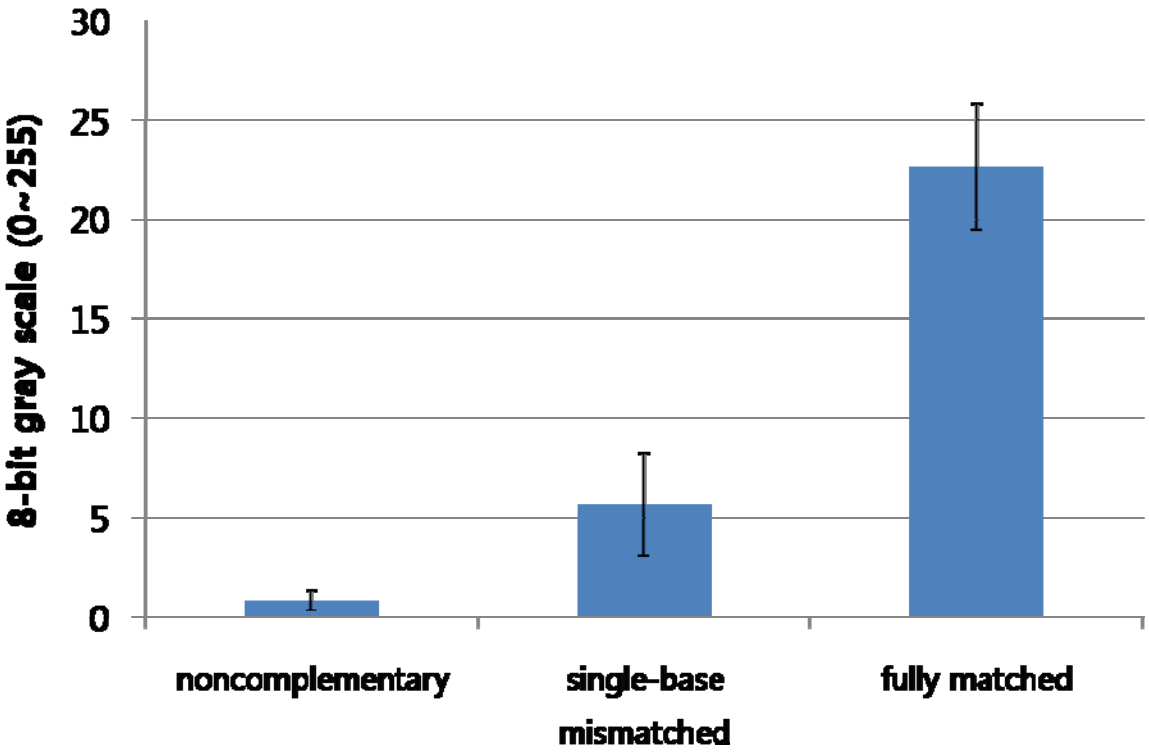

Figure S4. Surface hybridization of PNA capture probes to 1 nM fully matched DNA, single-base mismatched DNA (central mismatch), and non-complementary DNA in PBST buffer at 37℃. The single-base mismatched DNA showed lower intensity than the fully matched DNA.

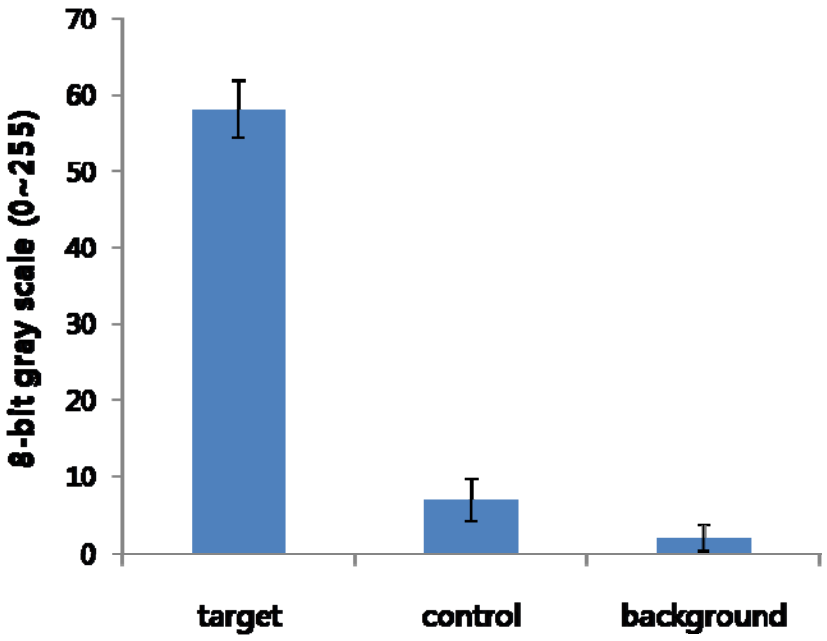

Figure S5. Graph of grayscale value depending on the hybridization mixture composition. Even in the presence of bulk DNA/RNA, our method could detect long target DNA.

(target: 2 nM 96-mer target DNA, 0.1 μg/μl control RNA (Neo pa RNA, 1.0 kb in length, Roche), and 100 nM random sequence DNA (target B, 50-mer) in PBST. Control: 0.1 μg/μl control RNA and 100 nM random sequence DNA)