Electronic Supplementary Material (ESI) for Chemical Communications

Scanometric Analysis of DNA Microarrays Using DNA Intercalator-Conjugated Gold Nanoparticles

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MATERIALS AND METHODS

Preparation of the oligonucleotide

All probe DNAs and labeled DNAs were prepared by Bioneer Co., Korea. Table S1 shows the sequences of each DNA probe, primer and the complementary target DNA.

Hemagglutinin probes of the influenza A virus were prepared according to previously reported research. [1]

Preparation of daunorubicin to conjugated gold nanoparticles (DNR-AuNPs)

We prepared carboxylate-modified ~3 nm gold nanoparticles in aqueous solution using a previously reported method[2] with slight modifications. $HAuCl_4 \cdot 3H_2O$ (500 μL, 10 mM, in distilled water (DW)) was added to 20 mL of DW containing 100 μL of mercaptosuccinic acid (10 mM, DW), 11-mercaptoundecanoic acid (10 mM, ETOH), and 16-mercaptohexadecanoic acid (10 mM, ETOH). After 1 minute, 500 μL of $NaBH_4$ (100 mM, DW) was added immediately with vigorous stirring and the solution was allowed to sit for 3 hr. The color of the solution changed to a transparent dark brown. [2-4]. The completed COOH-AuNPs were washed three times using 50 K centrifugal

filters (MILLIPORE) with 0.2% Tween 20 (Fig S1). To fabricate the daunorubicin conjugated to gold nanoparticles, we used EDC/NHS coupling that can bind covalently with COOH-AuNPs and amine groups of DNR. Furthermore, to increase hydrophilicity into the DNR-AuNPs, we added ethanol amine with five times the amount of DNR concentration.

400 μL of a 0.1 M EDC/NHS solution (1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide/N-hydroxysulfosuccinimide (Sulfo-NHS), 80 μL of 50 mM ethanolamine, and 80 μL of 10 mM daunorubicin were mixed with 20 mL of the gold nanoparticle solution (0.2% Tween 20) for 2 hr. Finally, the DNR-AuNPs were purified using PD-10 columns (GE Healthcare).

Preparation of DNA arrays

Surface modification of a slide glass chip was completed using the following substances in order: Piranha solution (H_2O_2:H_2SO_4 = 1:3, 30 min), 3-aminopropyltrimethoxysilane (APTMS, 2%, ethanol, 2 hr), succinic anhydride (1M, DMSO) and ethyl (dimethylaminopropyl) carbodiimide (EDC)/N-hydroxysuccinimide (NHS) (0.4 M / 0.1 M, 1 hr). Next, an amine-modified DNA probe (10 μM, 1X saline-sodium citrate (SSC) buffer, 0.02% Sodium dodecyl sulfate (SDS)) was spotted using a microarrayer machine (Proteogen/Korea), and the chip was incubated for 1 hr. Finally, the arrays were blocked with ethanolamine (1 M, pH 8.5)[5].

Amplified target DNA preparation

The RNA of the influenza A virus (H1N1/A/Puerto Rico/8-V24/1934: seasonal influenza virus, H1N1/A/California/04/2009: novel swine-origin influenza virus) was purified using an RNA purification KIT (QIAGEN). After preparation of the cDNA with a cDNA KIT (Roche), we performed an

asymmetric polymerase chain reaction (PCR) to prepare single-stranded target DNA. Each PCR reaction was conducted using a 0.8 μM forward and an 80 pM reverse primer. The reaction mixture was incubated for 10 min at 94°C. PCR (40 cycles) was performed at 94°C for 30 sec, 50°C for 30 sec, and 72°C for 20 sec followed by incubation at 72°C for 2 min. The Cy3-labeled ssDNA was prepared by adding a given concentration of dUTP-Cy3 to the DNTP mixture [1]. The products were purified using PCR product purification KIT (Bioneer/Korea). The ssDNA lengths for the seasonal influenza virus and swine-origin influenza virus were 1163 mer and 1158 mer, respectively.

Target DNA detection

The DNA chips were hybridized in solutions containing target DNA (5× SSC, 0.2 % SDS) at 37°C for 1 hour with slight shaking. When using amplified ssDNA for the influenza A virus (H1N1/hemagglutinin partial region), a hybridization reaction was performed in a hybridization chamber (Agilent) with 0.1 mL of hybridization solution containing 5XSSC and 0.2% Tween 20 at 52 °C for 6 hr. Following hybridization, the arrays were washed with 2XSSC containing 0.1% SDS at room temperature for 30 min. After the glass was thoroughly washed, a solution containing 1 mL of the DNR-AuNPs was dropped onto the arrays and incubated for 10 min at room temperature. After washing with 2XSSC containing 0.1% SDS, the array with a gold enhancement solution (Nanoprobes, USA) for 10 min. After washing with distilled water and drying with N_2 gas, optical images were obtained using an optical flatbed 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 using Photoshop (Adobe Systems). The fluorescent images were obtained with a fluorescent scanner (Genefix 4200A, Molecular Devices) set at 400 PMT and 70% power.

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Supplementary Information

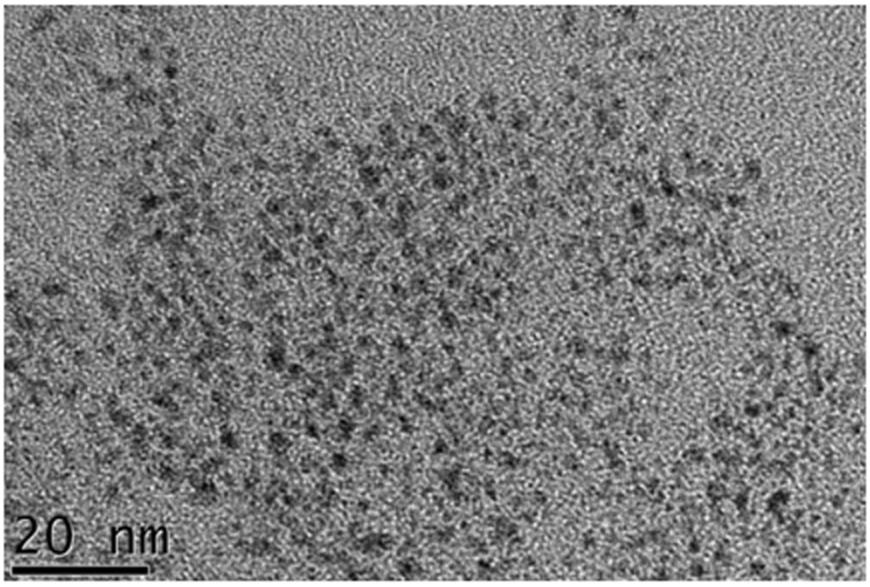

Fig. S1. A TEM image of the carboxylate-modified gold nanoparticles (~3 nm).

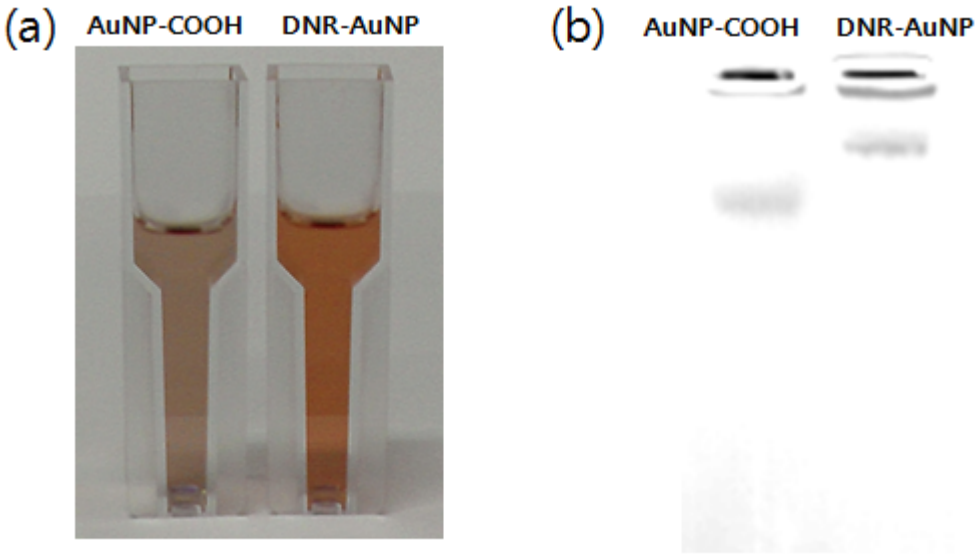

Fig. S2. (a) AuNP-COOH and DNR-AuNP after purification with PD-10 columns. (b) Electrophoresis of AuNP-COOH and DNR-AuNP. (c) Verification of covalent coupling (AuNP-COOH and daunorubicin) using zeta potential measurement.

Solution for gold nanoparticles dispersion	Zeta potential (mV)
AuNP-COOH	-13.85 ± 0.59
DNR-AuNP	-4.51 ± 0.18

Table S1. Zeta potential values of the AuNP-COOH and DNR-AuNP in 0.2% Tween 20.

Hemagglutinin probes of Influenza A H1N1			
H1-SW-1	GCTGGATCTGGTATTATCATT	H1-SE-1	ACAGTGACACACTCTGTCAAC
H1-SW-2	GGAGCAAAAAGCTTCTACAAA	H1-SE-2	TCAGTGTCATCATTTGAAAGA
H1-SW-3	TCCTCATGCTGGAGCAAAA	H1-SE-3	ACCGACACTGTTGACACAGTA
H1-SW-4	AAAAGCACAAAATTGAGACTG	H1-SE-4	GATTATGAGGAATTAAGGGAG
H1-SW-5	CAGCAAATCCTACATTAATGATAA	H1-SE-5	ACAGACACTGTTGACACAGTA
H1-SW-6	TGTGAATCACTCTCCACAG	H1-SE-6	CACCCAGTCACAATAGGAGAG
H1-SW-7	AGTTCAGACAATGGAACGTGT	H1-SE-7	GAGAATGGAACATGTTACCCA
H1-SW-8	AGCTTCTACAAAAATTTAATATGG	H1-SE-8	AACTACTACTGGACTCTGCTG
H1-SW-9	TCGAACAAAGGTGTAACGG	H1-SE-9	CATCCAGTCACAATTGGAGAA
H1-SW-10	TACATTGTGGAAACATCTAGTTC	H1-SE-10	GAAAATGGAACATGCTACCCC
H1-SW-11	TCATGGCCCAATCATGAC	H1-SE-11	AACTATTACTGGACCTTGCTA
H1-SW-12	CAACCGCAAATGCAGACA	H1-SE-12	TGGATCTTAGGAAACCCAGAA
H1-SW-13	TTCAAGCCTGAAATAGCAATA	H1-SE-13	GACTATGAGGAACTGAGGGAG
Primers		**Target**	
H1-SW-F	AGCAAAAGCAGGGGAAAATA	**H1-SW-1 Target**	AATGATAATACCAGATCCAGC
H1-SW-R	TCCTGACCCCTGCTCATTTT	**Other subtype probes**	
H1-SW-F	GGTCCTGTTATGTGCACTTG	**H2-3**	CCATCATTCTTCAGGAACATG
H1-SW-R	GTAATCCCGTTAATGGCATT	**H3-2**	AGCTCAATAATGAGGTCAGAT
Single base mismatched probe		**H4-3**	GCATGCAAAAGAGCAAATGTG
H1-SW-1-SM	GCTGGATCTGTTATTATCATT	**H5-7**	GAGAGTAATGGAAATTTCATT

Table S2. Oligonucleotide sequences of probes, primers and synthetic target DNA used in this research. All probes were modified by C6 amine at 3′.

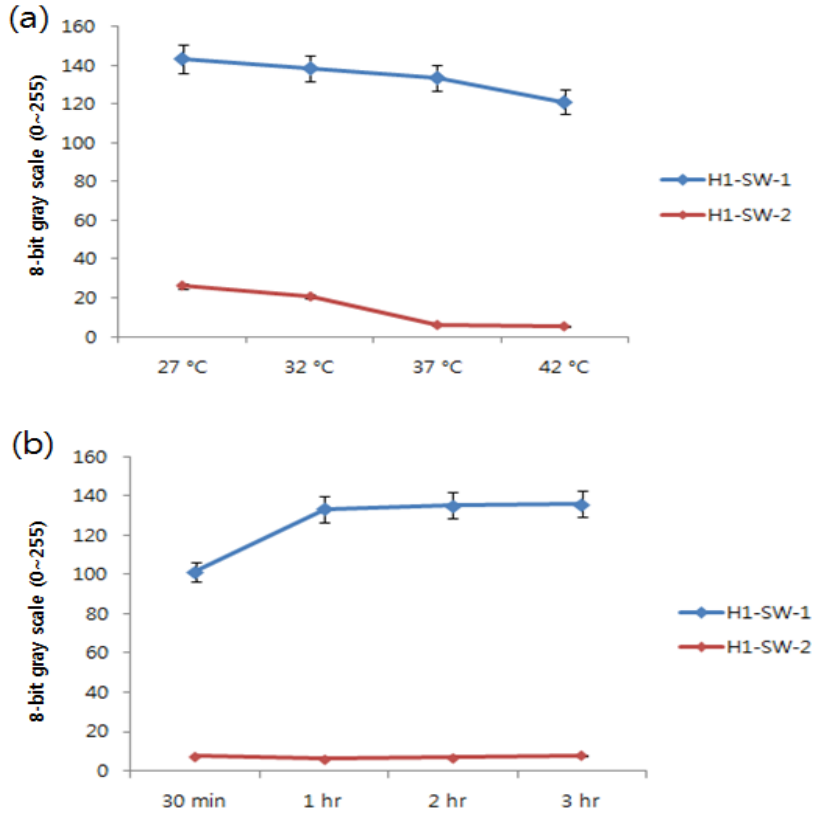

Fig S3. Optimization of hybridization temperature (a) and time (b) with 10 uM H1-SW-1 synthetic target DNA.

(Hybridization temperature optimization was performed for 1hr (a) hybridization time was optimized at a temperature of 37 °C (b). Arrays immobilized with H1-SW-1 and H1-SW-2 probes were used.)

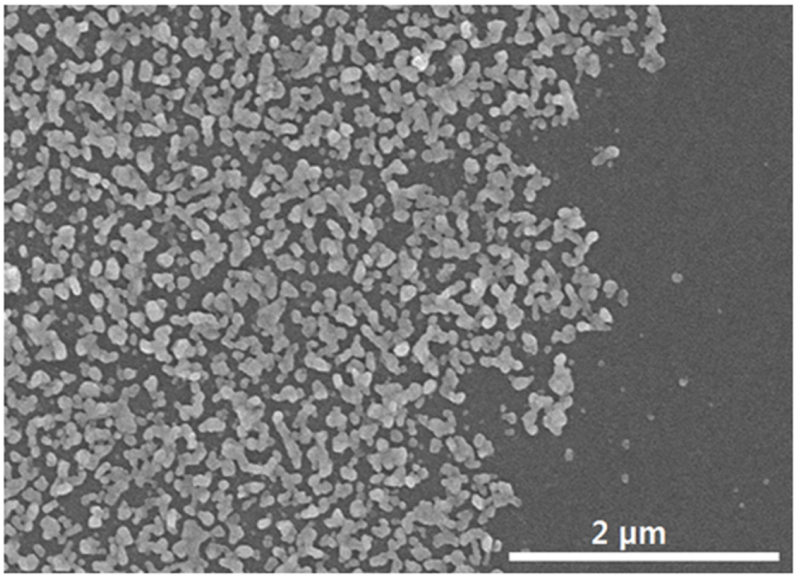

Fig S4. SEM image of DNR-AuNPs following gold enhancement process.

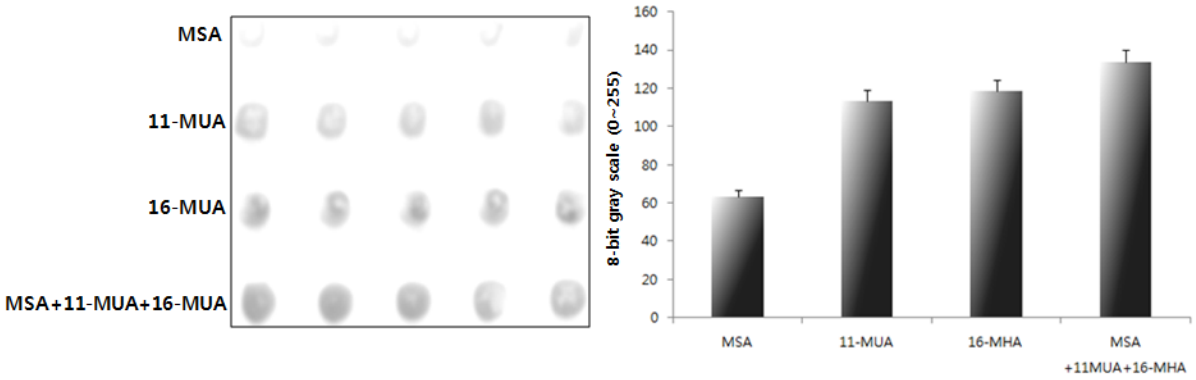

Fig S5. The results of the 8-bit grayscale values with different lengths of spacers on the DNR-AuNPs. The effect of the spacer length with MSA, 11-MUA, 16-MHA, and mixed samples were tested. When using only MSA conjugated AuNPs, the signal is lower than other samples. Although, 11-MUA and 16-MHA conjugated AuNPs have high intensity, mixed samples show the highest intensity.

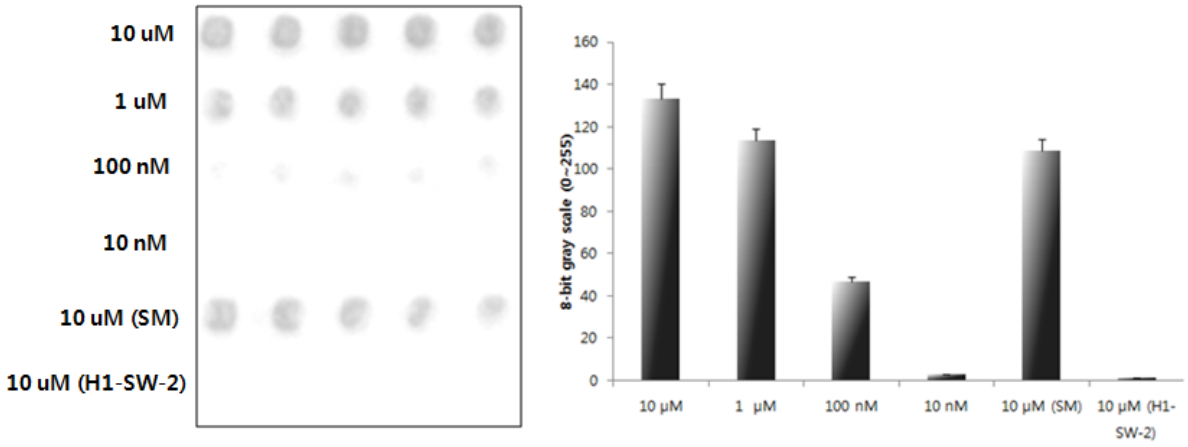

Fig S6. The results of the 8-bit grayscale values with different concentrations of probe (H1-SW-1) and a single-mismatched probe (H1-SW-1-SM) against 10 nM H1-SW-1 synthetic target DNA. The intensity is proportional to the concentration of the probe DNA within a range of 10 μM to 10 nM. The spots of a single mismatched probe (SM) show a slightly reduced intensity compared to the perfectly matched spots (control probe DNA with H1-SW-2 were used).

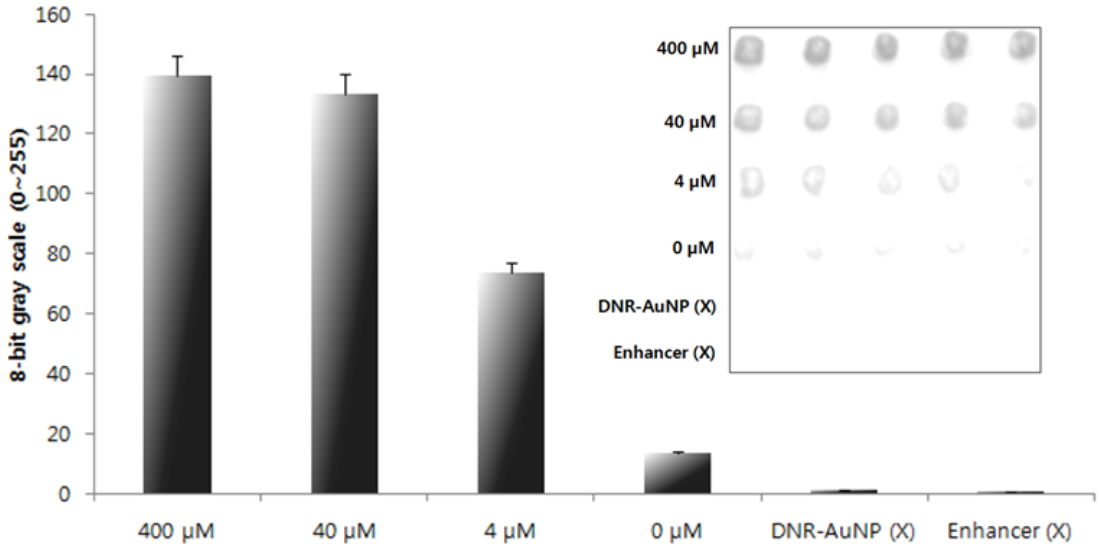

Fig S7. The results of the 8-bit grayscale values with different DNR concentration. The intensity was proportional to the concentration of the DNR. When measured without DNR-AuNP and AuNPs enhancement, each spot showed similar intensity as a background signal.

Table 3. Comparison of DNR-AuNPs with previously reported methods

	Selectivity	Sensitivity	Reproducibility	Target labeling process	Cost	High-throughput
Electrical method	good	~ 10 pM	good	nothing	low	difficult
Naked-eye DNA chip (target labelling)	good	~ 50 fM	good	need	low	difficult
Naked-eye PNA chip	good	~ 10 pM	good	nothing	expensive	easy
Naked-eye DNA chip (DNR-AuNPs)	good	~ 10 pM	good	nothing	low	easy

(We compared with electrical[6], naked-eye method-target labelling[7], naked-eye method-PNA chip[5], naked-eye-DNA chip (this study)).

1. Ryabinin, V.A., et al., *Universal oligonucleotide microarray for sub-typing of Influenza A virus.* PLoS One, 2011. **6**(4): p. e17529.
2. Laaksonen, T., et al., *Stability and electrostatics of mercaptoundecanoic acid-capped gold nanoparticles with varying counterion size.* Chemphyschem, 2006. **7**(10): p. 2143-9.
3. Brown, K.R., D.G. Walter, and M.J. Natan, *Seeding of colloidal Au nanoparticle solutions. 2. Improved control of particle size and shape.* Chemistry of Materials, 2000. **12**(2): p. 306-313.
4. Chen, H., et al., *Shape- and size-dependent refractive index sensitivity of gold nanoparticles.* Langmuir, 2008. **24**(10): p. 5233-5237.
5. Kim, S.K., et al., *Label-free and naked eye detection of PNA/DNA hybridization using enhancement of gold nanoparticles.* Chem Commun (Camb), 2010. **46**(19): p. 3315-7.
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7. Taton, T.A., C.A. Mirkin, and R.L. Letsinger, *Scanometric DNA array detection with nanoparticle probes.* Science, 2000. **289**(5485): p. 1757-60.