Supporting Information

Development of Label-Free Colorimetric Assay for MERS-CoV Using Gold Nanoparticles

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Figure S1. Native polyacrylamide gel electrophoresis (PAGE) analysis for confirmation of disulfide induced self-assembly with target DNA and probes in Figure 3. (M: DNA ladder, lane 1: disulfide induced self-assembly with ORF1a templates and probes, lane2: disulfide induced self-assembly with upE templates and probes, lane 3: non-extended result of TMV templates with mismatched ORF1a probes.) Note that lane 1 and lane 2 indicate positive reaction and lane 3 indicates negative reaction, respectively.
**Figure S2.** Agarose gel electrophoresis analysis for size confirmation of disulfide induced self-assembly (dsDNA) and ssDNA of target DNA and probes. (a) Visualizing separated DNA fragments during electrophoresis and (b) The full-length electrophoresis images of (a). (M: DNA ladder, lane 1: disulfide induced self-assembly with upE templates and probes, lane 2: upE templates, lane 3: 5'-upE probes, lane 4: 3'-upE probes, lane 5: TMV templates with ORF1a probes, lane 6: disulfide induced self-assembly with orf1a templates and probes, lane 7: ORF1a templates, lane 8: 5'-orf1a probes, lane 9: 3'-ORF1a probes)
Figure S3. Hydrodynamic size distribution measured by dynamic light scattering (DLS) for gold nanoparticles reacted with ssDNA of negative target DNA and mismatched probes (blue) or disulfide induced self-assembly (dsDNA complex) (green). The average hydrodynamic size of the gold nanoparticles, which was measured by dsDNA from the positive reaction, was increased by approximately 8.11 nm compared to immobilized ssDNA on gold nanoparticles from the negative reaction.
Overall experimental materials and methods
Gold(III) chloride trihydrate (520918, ≥99.9% trace metal basis, HAuCl₃, 3H₂O), and trisodium citrate dehydrate (S4641, ≥ 99.9% ACS reagent) were purchased from Sigma Aldrich (St. Louis, USA). In our experiments, HAuCl₃ and trisodium citrate dehydrate were used as a precursor for the preparation of gold nanoparticles (AuNPs) and as a reducing agent as well as a capping agent that stabilizes AuNPs, respectively. As a target, the partial genomic size (30 bp) of upstream E protein gene (upE) and open reading frames (ORF) 1a related to Middle East respiratory syndrome coronavirus (MERS-CoV) were selected. Each of the probes were thiolated at the 5′ site (Right: 5′R) or 3′ site (Left: 3′L). All synthesized oligonucleotides were purified by PAGE and confirmed with mass spectrometry (Bioneer Co., Seoul, Korea).

Native polyacrylamide gel electrophoresis (PAGE) analysis was carried out using 20% TBE gel (Novex™ TBE Gels, Invitrogen, CA, USA) in a 1x tris-borate EDTA buffer (Biosesang Inc., Korea) with overnight running conditions at 30 V. For DNA staining, EvaGreen® dye (GreenLight™ safe gel stain, Bioassay, Daejeon, Korea) was used and the stained gel image was captured along with Gel Doc™ XR+ (Bio-Rad, CA, USA).

Additional gel electrophoresis was performed with 4% agarose gels made by four of MIDORIGreen Advance TBE Agarose Tablets (Nippon Genetics Europe GmbH, Germany) with pure water.

Preparation of Gold Nanoparticles: In our experiments, the synthesis of AuNPs was performed under a fixed concentration of HAuCl₃. Briefly, 50 mL of a 0.01 wt% gold(III) chloride trihydrate (HAuCl₃) solution was boiled, then varied amounts of 1% sodium citrate tribasic dehydrate solution (300, 500, 1200 and 1600 µL, respectively) was quickly added under stirring. After a while, an orange or red colored aqueous AuNPs solution was obtained. Subsequently, the aqueous solution was continuously boiled for a few minutes and cooled slowly to room temperature. Samples were used without further purification.
**Preparation of the Templates and ssDNA Probe:**

Each of the probes were thiolated at the 5’ site (Right: 5′R) and 3′ site (Left: 3′L). The sequences and description of all types of oligonucleotides used in our assay are listed in Table S-1.

Table S-1. List of synthesized probes and template sequences used in this study

| Template | Left probe | Right Probe |
|----------|------------|-------------|
| ORF1a    | AGTAGTGGGCTCGTA [Thiol] | CGCTGACGAAATGGG |
| upE      | CATAGTACGCAGAG [Thiol] | CCTCTACACGGGACC |
| TMV      | GGAAGACCATACCGACCACGTACAGTTAGAT | - |

**Analyzed Instruments:**

The morphology of the prepared gold nanoparticles was verified through transmission electron microscopy (TEM). The samples for TEM analyses was placed as a colloidal solvent onto a carbon-coated copper grid and dried at room temperature. The images were obtained with a JEM-3010 TEM (JEOL Ltd., Tokyo, Japan).

The varying diameters of AuNPs were determined by dynamic light scattering (DLS) (Zetasizer Nano ZS; Malvern Instruments Ltd., Herrenberg, Germany) operating with a He-Ne laser at a wavelength of 633 nm using back scattered light. 1 ml samples were loaded in a cuvette and three measurements were performed for each run.

The UV-vis spectra were then measured using a BioSpectrometer® (Eppendorf, Hamburg, Germany). All spectra measurements were performed in triplicate.

X-ray photoelectron spectroscopy (XPS) (ESCALAB 250, Thermo Fisher Scientific, USA) with an AlKα X-RAY source and monochromator under ultrahigh vacuum (1 × 10⁻⁹ Torr) was carried out to confirm the presence of the sulfur-groups on gold surface. For XPS analysis the solution
was dropped onto a silicon wafer and dried overnight in air at room temperature. Silicon wafers were cleaned by sonication in acetone and isopropanol for five minutes each before use.

**Data analysis and statistics:**

The spectral centroid of the absorbance spectrum is calculated as follows:

\[
\lambda_{CM} = \frac{I_1 \lambda_1 + I_2 \lambda_2 + I_3 \lambda_3 + \cdots + I_n \lambda_n}{I_1 + I_2 + I_3 + \cdots + I_n} = \frac{\sum_{i=1}^{n} I_i \lambda_i}{\sum_{i=1}^{n} I_i}.
\]

(\(\lambda_{CM}\): the spectral centroid of the absorbance spectrum; \(I\): the intensity of the spectrum; \(\lambda\): the wavelength; \(n\): pixels that are contained in the calculation.)