This journal is © The Royal Society of Chemistry 2013

**Electronic Supporting Information**

for

# Detection of DNA Induced Gold Nanoparticle Aggregation with Dark Field Imaging†

**Tong Bu[a,b], Tamotsu Zako*[,b] Masahiro Fujita[b], and Mizuo Maeda*[,a,b]**

[a]*Bioengineering Laboratory, Department of Advanced Materials Science, School of Frontier Sciences, The University of Tokyo, Kashiwa, Chiba 227-8561, Japan,*

[b]*Bioengineering Laboratory*, RIKEN, *Wako, Saitama 351-0198, Japan*.

E-mail:zako@riken.jp, mizuo@riken.jp

Fax: (+81)-48-462-4658; Tel: (+81)-48-467-5489

**Table of Contents**

**Experimental** S2

**Fig. S1.** Colour changes (a) and UV-VIS spectrum (b) of ssDNA-AuNP solution after the addition of the complementary DNA S5

**Fig. S2**. Intensity histogram of AuNPs aggregates observed by dark field microscopy (DFM) S6

This journal is © The Royal Society of Chemistry 2013

## Experimental

### Materials

All the oligonucleotides were purchased from Operon Japan. The DNA sequences were shown as below. The concentration of each strand was estimated by measuring $OD_{260}$.

Probe DNA : 5’-Thiol-$(CH_2)_6$ -TACGCCACCAGCTCC- 3’
Complementary ssDNA : 5’-GGAGCTGGTGGCGTA-3’
Mismatched ssDNA : 5’-TGAGCTGGTGGCGTA-3’

The gold nanoparticles (AuNPs, 40 nm) was purchased from British Biocell international. The concentration of the AuNP was calculated using the manufacture’s coefficient data ($\varepsilon_{520}$ (40 nm) = $9.0 \times 10^{10}$ $cm^{-1}$).

### Functionalization of gold nanoparticles with thiolated DNA

The functionalization of AuNPs with thiolated probe DNA follows the procedure as previously described (ref, K. Sato, K. Hosokawa and M. Maeda, *J. Am. Chem. Soc.*, 2003, **125**, 8102.). Three nmol of probe DNA was incubated with 1 mL of the AuNP solution at 50℃ for 16 h. The solution was changed into 0.1 M NaCl, 10 mM phosphate buffer (pH 7) by addition of the necessary salts and was kept at 50 °C for 40 h. To remove unreacted probe DNA, the solution was centrifuged at 15000 rpm for 20 min, then the supernatant was replaced by 1 mL of the same buffer. After another centrifugation, the precipitate was re-dispersed into 0.3 mL of the same buffer to make a stock solution.

### Estimation of the amount of the immobilized probe DNA

To release the immobilized probe DNA from AuNPs, 10 mM dithiothreitol (DTT) with was added to the ssDNA-AuNP solution. The solution was incubated at room temperature for 16 h. After centrifugation at 14000 rpm for 25min, the concentration of released probe DNA in the supernatant was quantified using OliGreen ssDNA Quantitation Kit (Invitrogen).

### DNA-induced AuNPs aggregation

Various concentrations (from 0 to 250 nM) of complementary ssDNA (target DNA) were added into the ssDNA-AuNP solution ($1.98\times10^{10}$ $mL^{-1}$ (=32 pM)) in the presence of 1 M NaCl, and incubated for 1h at room temperature. Mismatched ssDNA (100 nM) was added as a negative control sample.

## Dark field microscopy (DFM) analysis

For the DFM analysis, 6 μL of the AuNPs samples were deposited onto glass slides (High Density Amine Coated Slides, Matsunami Glass Ind., Osaka, Japan) and covered with coverglass. DFM images were taken using BX53 microscope (Olympus, Tokyo, Japan) equipped with U-DCW dark field condenser, UPlanFLN 60×objective lens and DP73 CCD camera (Olympus). Images were taken using CellSens Standard software version 1.6 (Olympus). AuNPs were identified as bright orange-coloured spots in DFM images and the scattered light intensity of each spot was quantified. The brighter spots that have intensities higher than 24 were defined as aggregates, since 95% of the dispersed negative control sample to which 100 nM of mismatched ssDNA was added showed an intensity lower than 24. The ratio of aggregates at each target DNA concentration was estimated to calculate the LOD.

## Calculation of LOD

The LOD was evaluated by the 3σ criterion method, where σ denotes the standard deviation of zero-concentration background data (n=6). Average values from three histograms obtained by three different glass slides were shown in the figure. The data points of the calibration curve were fitted with a four-parameter logistic function (ref, C. T. Diamandis, "Immunoassay" Academic Press, 1996; H. Arata, H. Komatsu, K. Hosokawa and M. Maeda, PLoS One, 2012, **7**, e48329): $y=d+(a-d)/[1+(x/c)^b]$, where $a$, $b$, $c$ and $d$ are fitting parameters. These parameters were optimized by nonlinear least-square regression weighted by the reciprocal of the square of standard deviation of each datum point. The LOD was calculated from the fitting equation as the concentration that gives higher value than 3σ line (=background (zero-concentration) + 3σ).

## UV-Vis Spectrometer, Dynamic Light Scattering (DLS) and colour change

Conventional methods such as UV-VIS spectroscopy, DLS and colour changes were used to estimate LOD for comparison. Cary 50 UV-Vis spectrometer (Varian, Palo Alto, USA) was employed to measure UV-VIS absorption spectrum. The ratio of absorbance values at 630 and 530 nm ($A_{630}/A_{530}$) at various concentrations of added target ssDNA was used for estimation of aggregation of the AuNP solution. LOD was determined by the concentration of added ssDNA that gives higher $A_{630}/A_{530}$ value than 3σ line. Sample with addition of 100 nM mismatched DNA was used as negative control. Average values from three different samples were shown (n=6 for zero-concentration).

The hydrodynamic diameters of the AuNPs under investigation were measured using a dynamic light scattering (DLS) system (Malvern, ZetasizerNano ZS90, Worcestershire, UK). A Zen 2112 cuvette was used as sample container. Malvern software (DTS ver. 5.10) was used to analyze the data. The size of aggregates was obtained using a non-negative lease squares (NNLS) analysis methods based on intensity average. LOD was determined by the concentration of added ssDNA that gives larger size of aggregates than 3σ line. Sample with the addition of 100 nM

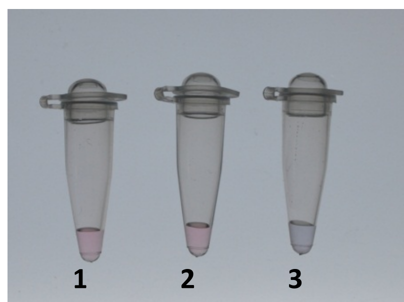
mismatched DNA was used as a negative control. The average values of three different samples were used (n=6 for zero-concentration). The photo-images of tubes were taken by digital camera.

**Time course assay**

For time course-assay, 50 nM of complementary ssDNA was added to the ssDNA-AuNP solution in the presence of 1 M NaCl. At different incubation time (0 to 120 min), sizes of the aggregates were measured by DLS. Also, 10 μL of colloid solution was taken at each time-point and was deposited adsorbed on glass slides for DFM imaging. The intensities of the each AuNP spot in the DFM image were determined, and the ratio of monomers (AuNP spots that have intensities less than 24) per all the spots at each time-point was estimated.

Electronic Supplementary Material (ESI) for Chemical Communications
This journal is © The Royal Society of Chemistry 2013

a




1 ssDNA-AuNP
2 +mismatched ssDNA (500 nM) (negative control)
3 +complementary ssDNA (500 nM)

b

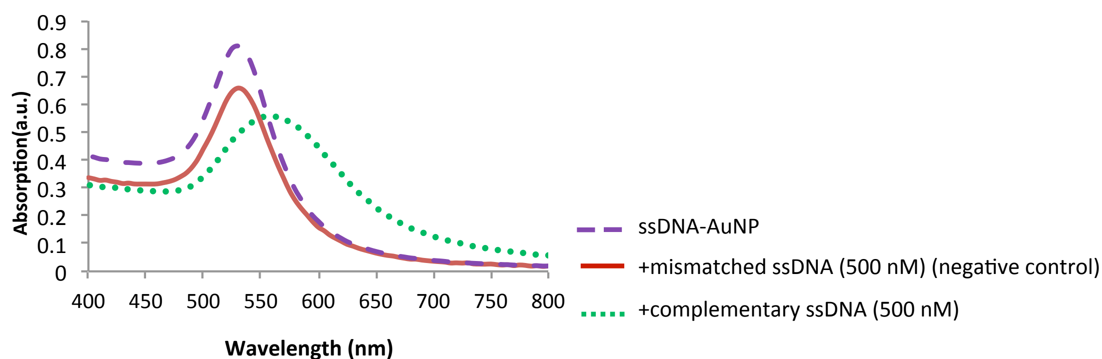



**Figure S1** a, Color changes of ssDNA-AuNP solution after the addition of the complementary DNA (500 nM) or mismatched ssDNA (500 nM, negative control) in the presence of 1 M NaCl and the following incubation for 3 min. b, UV-VIS spectrum of ssDNA-AuNP solution after the addition of the complementary DNA (500 nM) or mismatched ssDNA (500 nM, negative control) in the presence of 1 M NaCl and the following incubation for 3 min.

This journal is © The Royal Society of Chemistry 2013

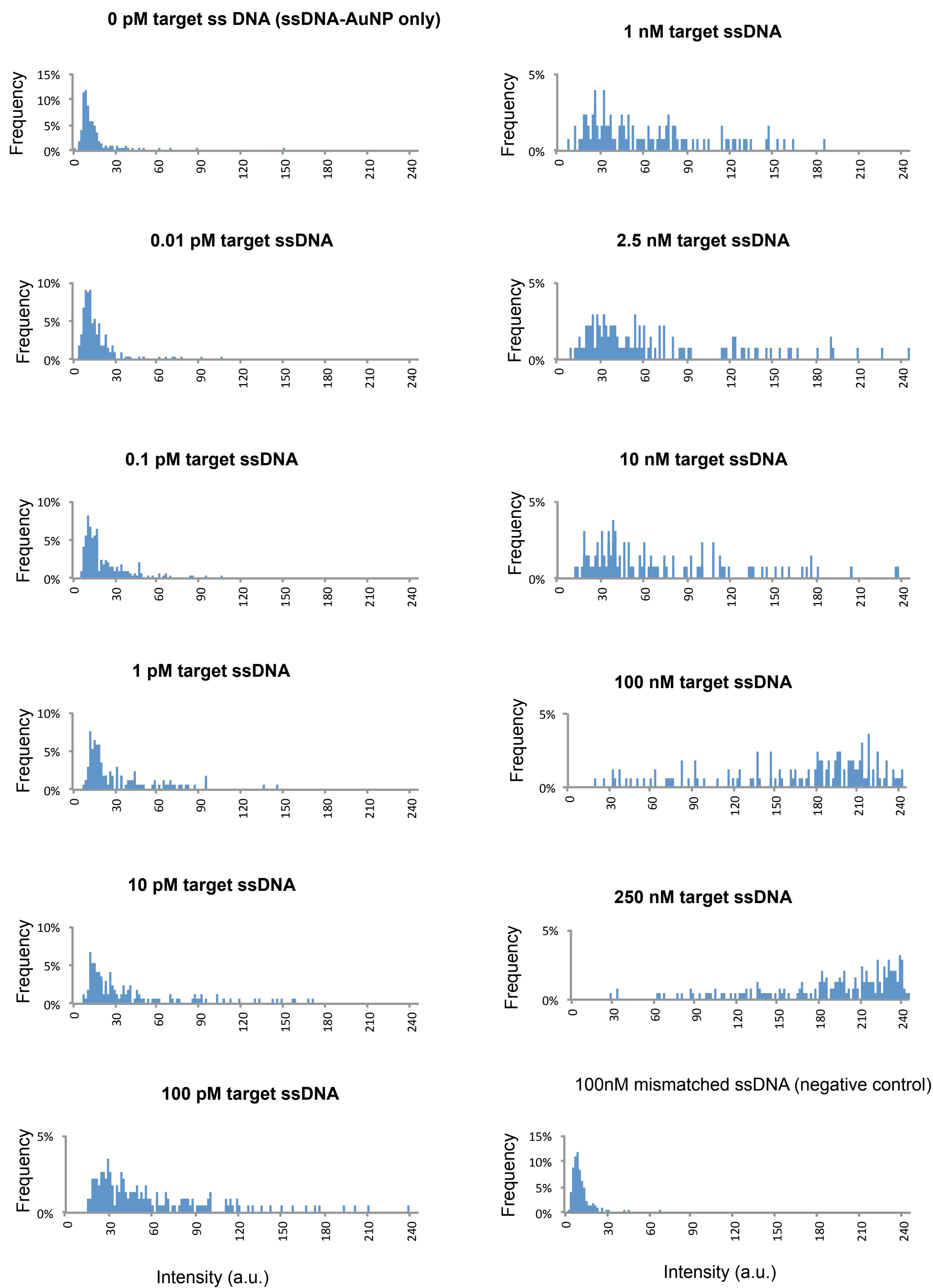



**Figure S2** Intensity histogram of AuNPs aggregates observed by DFM caused by adding different concentration of the target complementary ssDNA ranging from 0 to 250 nM (n>200). Mismatched ssDNA (100 nM) was added as a negative control.