Electronic supplementary information (ESI)

Rapid naked-eye detection of mercury ion based on non-crosslinking aggregation of double-stranded DNA-carrying gold nanoparticles

Naoki Kanayama,[†] Tohru Takarada,[*] and Mizuo Maeda

Bioengineering Laboratory, RIKEN Advanced Science Institute, Wako, Saitama 351-0198, Japan.

* E-mail: ttkrd@riken.jp; Fax: (+81)-48-462-4658; Tel: (+81)-48-467-5489

[†] Current Address: Graduate School of Pure and Applied Sciences, University of Tsukuba, Tannoudai 1-1-1, Tsukuba, Ibaraki 305-8573, Japan.

Contents

S-1 Experimental

General

All reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan) unless otherwise noted. A colloidal solution of gold nanoparticles (GNP) with a diameter of 40 nm was purchased from BBinternational (Cardiff, UK). The GNP concentration was 9.0×10^{10} mL^{-1}. The OPC-purified single-stranded DNA (ssDNA) and 5'-mercaptohexyl ssDNA (ssDNA-SH, reduced by dithiothreitol treatment) were purchased from Operon Biotechnologies (Tokyo, Japan). The ssDNA-SH was further purified with ethanol precipitation before use. The DNA concentration was determined by measuring the absorbance at 260 nm with a Cary 50 UV-Vis spectrophotometer (Varian Inc., Palo Alto, CA). Deionized water (> 18.1 MΩ·cm) purified with a Milli-Q instrument (Millipore, Billerica, MA) was used for all of the experiments.

Preparation of dsDNA-GNP

First, ssDNA-SH was grafted onto the GNP surface through the Au–S bond formation by following our previous procedure.[1] Briefly, 5 nmol of ssDNA-SH was incubated with 1 mL of the GNP dispersion at 50°C for 16 h. The dispersal medium was then exchanged for 10 mM phosphate buffer (pH 7.4) containing 0.1 M NaCl by directly adding the corresponding salts into the dispersion. The mixture was incubated at 50°C for 40 h. To remove unreacted ssDNA-SH, the solution was centrifuged at 14,000 rpm for 25 min, and the supernatant was replaced by 1 mL of 10 mM phosphate buffer (pH 7.4) containing 0.1 M $NaNO_3$. The precipitate was re-dispersed by vortex mixing for several seconds. This process was repeated three times. Finally, the precipitate was re-dispersed into 0.1 mL of the same buffer. Then, the dispersion was mixed with 200 μL of ssDNA solution (complementary ssDNA or its single-base-substituted mutant; 10 μM) to form a dsDNA layer on the GNP surface. After incubation at room temperature for 10 min, the resulting dsDNA-GNP dispersion was used for the following experiments.

Colloidal stability of dsDNA-GNP with or without Hg^{2+}

The dsDNA-GNP dispersion (15 μL) prepared as described above was mixed with 1 μL of milliQ water and 4 μL of 5 M $NaNO_3$ solution. The final concentration of $NaNO_3$ was 1 M. After incubation of the mixture at room temperature for 5 min, pictures of the mixture were taken with a digital camera (CAMEDIA C-5050ZOOM; Olympus, Tokyo, Japan). For the detection of Hg^{2+}, the dsDNA-GNP dispersion (15 μL) was mixed with

1 μL of $Hg(ClO_4)_2$ solution and 4 μL of 5 M $NaNO_3$ solution. UV-Vis spectra of the mixture were taken with a Cary 50 UV-Vis spectrophotometer using a micro quartz cell.

Melting temperature measurements

The melting curves of dsDNA in 2.5 mM phosphate buffer (pH 7.4) containing 0.5 M $NaNO_3$ were obtained by measuring the change of absorbance (A) at 260 nm as a function of temperature with a UV-2550 spectrophotometer equipped with a TMSPC-8 temperature controller unit (Shimadzu, Kyoto, Japan). The concentrations of ssDNA and $Hg(ClO_4)_2$ were 3 μM and 6 μM, respectively. The heating and cooling ramp was 1^oC/min. The sample-holding chamber was continuously flushed with dry N_2 gas during the measurements. Relative absorbance, $RA = (A_{t^oC}–A_{0^oC})/(A_{60^oC}–A_{0^oC})$, was employed to normalize the thermally induced change in the absorbance of sample solution. The melting temperature (T_m) was determined as an average of the maximum values in the first derivative of the melting curves obtained from the heating and cooling processes.

S-2 Melting curve measurements

The base sequences of dsDNAs and their melting curves are shown in Fig. S1. The melting temperatures determined from the curves are summarized in Table 1. The analysis revealed that Hg^{2+} induced an increase in the melting temperature of the dsDNAs having a T–T mismatch at the penultimate and antepenultimate position (dsDNA2 and dsDNA3), while a slight change of T_m was observed with the fully matched dsDNA (dsDNAc) and the dsDNA having a T–T mismatch at the distal end (dsDNA1).

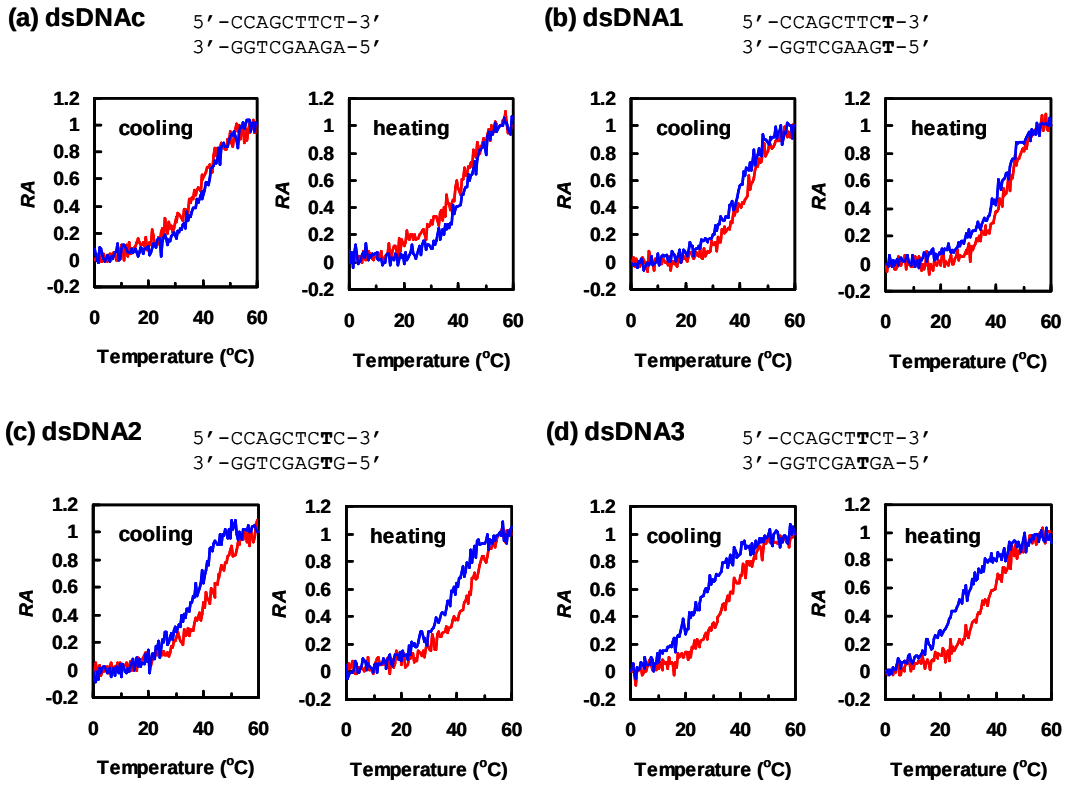

Fig. S1 Base sequences and melting curves for dsDNAs with (red) or without Hg^{2+} (blue) in 2.5 mM phosphate buffer containing 0.5 M $NaNO_3$.

S-3 Aggregation of GNP2 with Hg^{2+}

The colloidal stability change of GNP2 in the presence of $Hg(ClO_4)_2$ was examined. As shown in Fig. S2, the dispersion of GNP2 changed its color drastically from red to colorless within 1 min at a concentration of $Hg(ClO_4)_2$ above 1.0 μM.

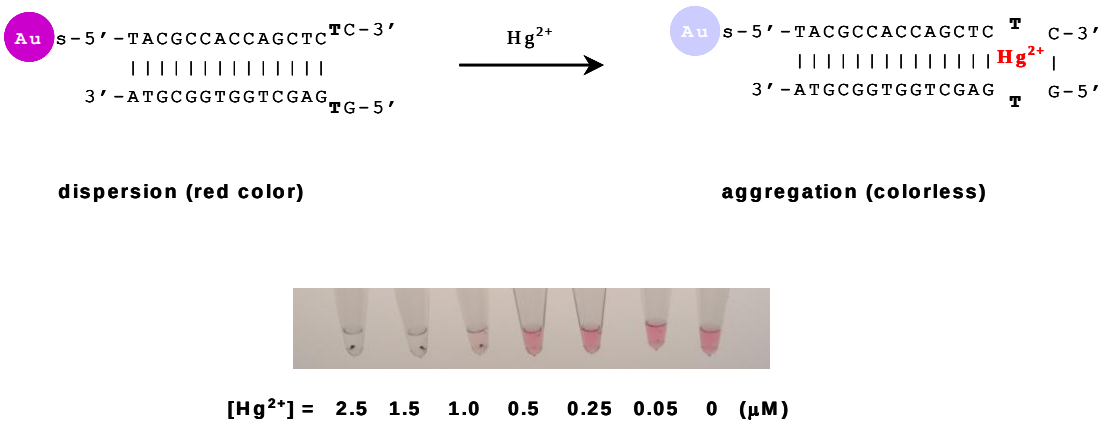

Fig. S2 Aggregation of GNP2 with $Hg(ClO_4)_2$ in the presence of 1 M $NaNO_3$.

S-4 No aggregation of GNP1 with Hg^{2+}

The colloidal stability of GNP1 in the presence of $Hg(ClO_4)_2$ was examined. As shown in Fig. S3, the dispersion of GNP1 showed a red color (no color change) even in the presence of 2.5 μM $Hg(ClO_4)_2$.

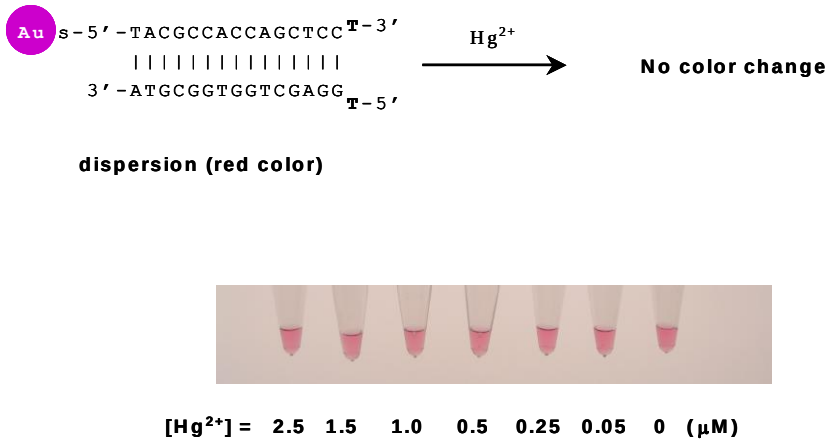

Fig. S3 No aggregation of GNP1 with $Hg(ClO_4)_2$ in the presence of 1 M $NaNO_3$.

S-5 Aggregation of dsDNA-GNP having a C–C mismatch with Ag^+

The colloidal stability change of dsDNA-GNP having a cytosine–cytosine (C–C) mismatch site at the antepenultimate position (see Fig. S4) in the presence of $AgNO_3$ was examined. As shown in Fig. S4, the dsDNA-GNP aggregated with 2.5 μM $AgNO_3$ in a non-crosslinking manner.

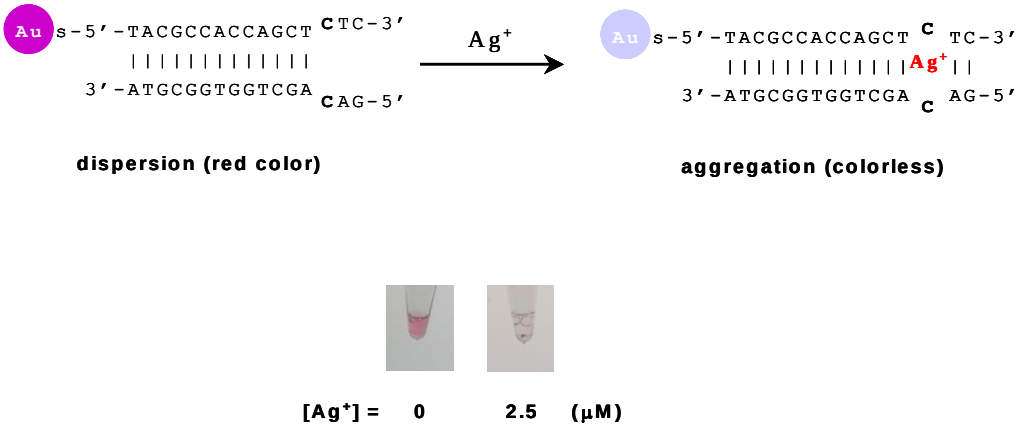

Fig. S4 Ag^+-induced aggregation of dsDNA-GNP having a C–C mismatch at the antepenultimate position in the presence of 1 M $NaNO_3$.

S-6 Aggregation of GNP carrying folded ssDNA with Hg^{2+}

The colloidal stability change of GNP carrying ssDNA with a hairpin-loop structure in the presence of Hg^{2+} was examined. The base sequence of hairpin-loop structure ssDNA was designed to have a T–T mismatch site at the penultimate position (see Fig. S5). As shown in Fig. S5, the dispersion of this GNP changed its color drastically from red to colorless within 1 min at a concentration of $Hg(ClO_4)_2$ above 1.0 μM.

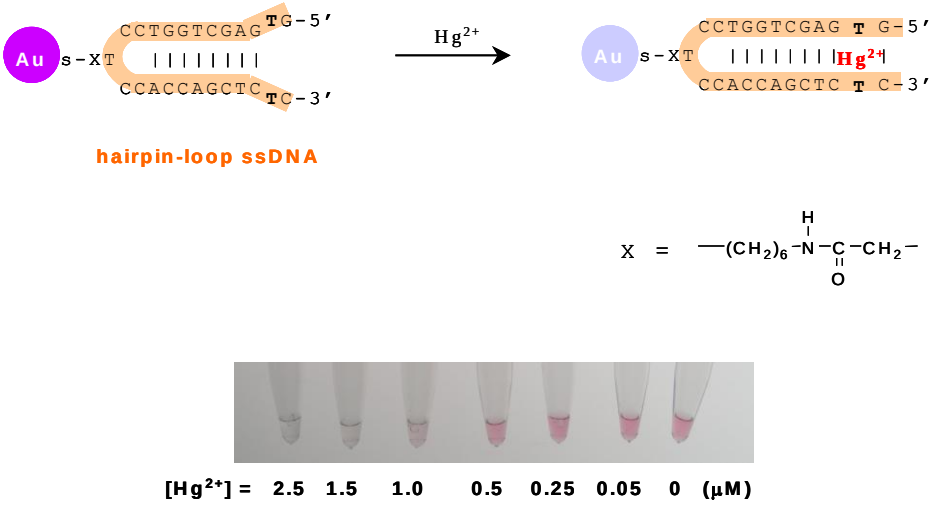

Fig. S5 Hg^{2+}-induced aggregation of GNP carrying ssDNA with a hairpin-loop structure.

Reference

1. K. Sato, K. Hosokawa and M. Maeda, *J. Am. Chem. Soc.*, 2003, **125**, 8102.