Supplementary Information

Plasmonic circular dichroism-based metal ion detection using gold nanorod–DNA complexes

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Experimental

1.1 Chemicals and Apparatus

20-(11-Mercaptoundecanyloxy)-3, 6, 9, 12, 15, 18-hexaoxaeicosane-1-amine (cationic ligand) hydrochloride was purchased from Dojindo Laboratories (Japan). Oligodeoxynucleotides (ODNs) were purchased from Thermo Fisher Scientific Inc. (USA). SYBR Green I was purchased from Lonza (Rockland, MD, USA). Metal standard solutions (Hg^{2+} and Ag^{+}, Cr^{6+}, Cu^{2+}, Fe^{3+}, Zn^{2+}) were purchased from FUJIFILM Wako Pure Chemical Co., Ltd (Japan). Extinction spectra were measured with a V-770 UV-vis spectrometer (JASCO Corp., Japan). Dynamic light scattering (DLS) profiles were obtained with a Zetasizer Nano ZS system (Malvern Panalytical, UK). The fluorescence images were captured using the Printgraph CMOS I (ATTO Corporation, Japan). Circular dichroism (CD) measurements were conducted with a J-820 spectrometer (JASCO Corp., Japan). Scanning transmission electron microscopic (STEM) images were obtained using a STEM HD-2000 system (Hitachi High-Tech Manufacturing & Service Co., Ltd., Japan) with a 200 kV acceleration voltage.

1.2 Synthesis of gold nanorods (AuNRs)

AuNRs with a diameter of ∼10 nm and a length of ∼40 nm were synthesized according to the seed growth method.[1] (1) Preparation of seed solution: a gold seed solution was prepared in a 50 mL plastic tube by adding an ice-cold $NaBH_4$ solution (600 μL, 0.01 M) to a mixture of a $HAuCl_4{\cdot}3H_2O$ solution (250 μL, 0.01 M) and a CTAB solution (7.5 mL, 0.1 M) under gentle shaking. The seed solution was then kept in an incubator (30°C) for at least 2 h. (2) Growth reaction: growth solutions were prepared in 100 mL plastic tubes by sequentially adding $HAuCl_4{\cdot}3H_2O$ solution (400 μL, 0.01 M), $AgNO_3$ solution (240 μL, 0.01 M) and an ascorbic solution (64 μL, 0.1 M) to a CTAB solution (40 mL, 0.1 M). The gold seed solutions (96 μL) were injected into the growth solutions (40 mL) while the growth solution was shaken gently. The growth solutions were then left undisturbed in an incubator (30°C) for at least 3 h. (3) Purification: the prepared AuNRs solutions were transferred to 1.5 mL plastic tubes and divided into 1000 mL portions. The AuNRs were sedimented by centrifugation (18,000 g, 20 min, 30°C), and the supernatant (900 μL) was removed. The plastic tubes were then filled with Milli-Q water (900 μL) and vortexed. This centrifugal purification procedure was repeated twice so that all chemicals except for the AuNRs were diluted 100-fold.

1.3 Surface modification of AuNRs with cationic ligands

Methanol solutions of the cationic ligand(20-(11-Mercaptoundecanyloxy)-3, 6, 9, 12, 15, 18-hexaoxaeicosane-1-amine, hydrochloride) (10 mM) were prepared. The ligand solution (10 mM, 35 μl) was added to the purified AuNRs solution (1000 μL) in a 1.5 mL plastic tube. The AuNRs solution containing the ligands was vortexed and kept undisturbed at 42°C for at least 12 h. Then, the solution was centrifuged (18 000 g, 20 min, 30°C), the supernatant (900 μL) was removed, and Milli-Q water (900 μL) was added to the residue solution. This centrifugal purification was performed twice to remove free ligands from the AuNRs solution. Finally, the concentration of the ligand modified AuNRs was adjusted to the desired concentration by centrifugal concentration.

1.4 Preparation of AuNR–ODN complexes

In a typical procedure, Tris-HCl buffer solution (20 μL, 100 mM, pH=7.6), Milli-Q water (53.6 μL), and Hg^{2+} solution (26.4 μL, 50 μM) were added to a 1.5 mL plastic tube. ODN was then added to this mixture and vortexed. Finally, cationic ligand-modified AuNRs (70 μL, 45.7 nM) were added and

vortexed again to prepare AuNR–ODN complexes.

1.5 Fluorescence measurement of SYBR Green I (SG)

In a typical procedure, Tris-HCl buffer solution (20 μL, 100 mM, pH=7.6), Milli-Q water (119.6 μL), metal ion solution (Hg^{2+}, Ag^{+}, Cr^{6+}, Cu^{2+}, Fe^{3+} and Zn^{2+}) (26.4 μL, 50 μM), ODN (30 μL, 4 μM), and SG (4 μL, 100×) were mixed in a 1.5 mL plastic tube and vortexed. Then, each solution was transferred to a quartz cuvette with an optical path length of 1 mm. Finally, the fluorescence intensity of each solution was measured with a Printgraph CMOS I system. The measurement conditions are as follows; filter: cyan, iris: 19, zoom: 0, focus: 3, and time: 4 sec.

1.6 Circular Dichroism (CD) measurement

Each of the samples was transferred to a quartz cuvette with an optical path length of 1 mm. The quartz cuvettes were set in a CD spectrometer, and the CD spectra were collected. The measurement conditions were as follow;, scan speed: 200 nm/min, response: 1 msec, bandwidth: 15 nm, and scans: 2. All of the CD spectra were smoothed using a 50-point Savitzky-Golay algorithm.

1.7 Calculation of dissymmetry factor (*g*-factor)

The dissymmetry factor (*g*-factor) is defined as:

$$g\text{-factor} = \frac{\Delta\varepsilon}{\varepsilon}$$

where Δε is molar circular dichroism and ε is molar absorption coefficient. From Beer-Lambert law, Δε and ε can be expressed as follows:

$$\Delta\varepsilon = \frac{\Delta A}{c \times l}$$

$$\varepsilon = \frac{A}{c \times l}$$

where ΔA is the difference between the absorbance of left and right circularly polarized lights, A is the absorbance of the sample, c is the concentration of the sample, and l is the optical path length of the cuvette. Thus, when absorption and CD spectra are measured at the same concentration with the same optical cuvette, the *g*-factor can be expressed as follows:

$$g\text{-factor} = \frac{\Delta A}{A}$$

The output of CD spectrometers is usually measured as ellipticity θ (in mdeg), related to ΔA through θ (mdeg) $=32980 \times \Delta A$. Therefore, the *g*-factor can be calculated as follows:

$$g\text{-factor} = \frac{\theta}{32980 \times A}$$

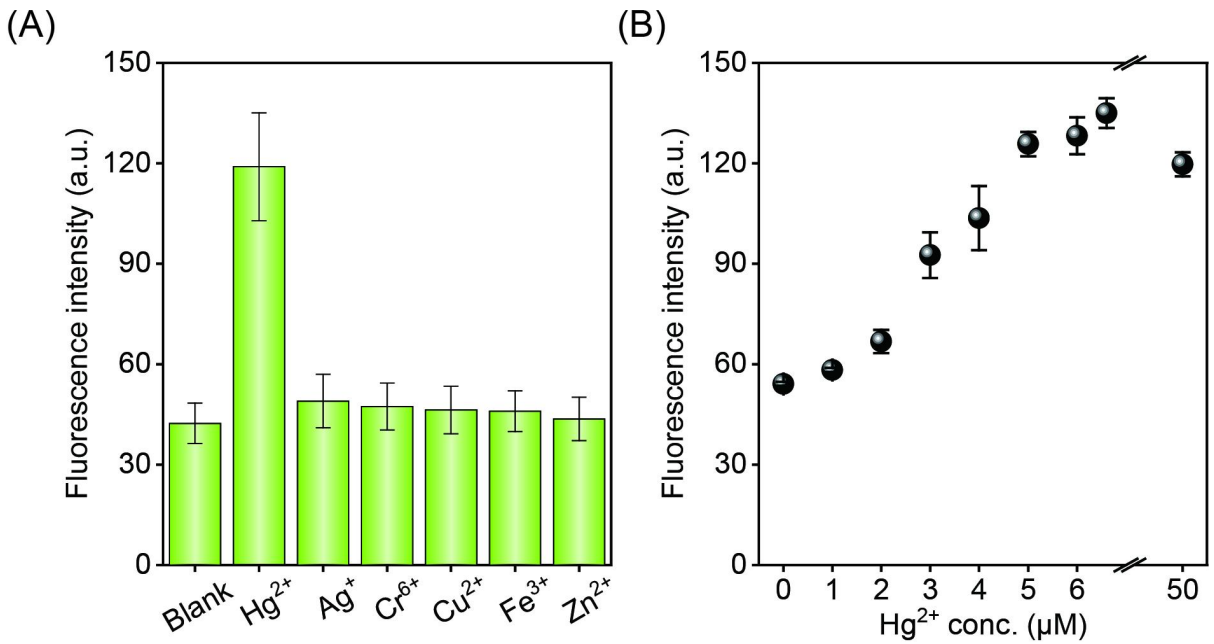

Figure S1 (A) The fluorescence intensity of a mixture of ODN_{dT22} and SG containing Hg^{2+} and other metal ions (Ag^{+}, Cr^{6+}, Cu^{2+}, Fe^{3+}, and Zn^{2+}). (B) Plots of the fluorescence intensity versus Hg^{2+} concentration. Error bars represent standard deviation (n = 3).

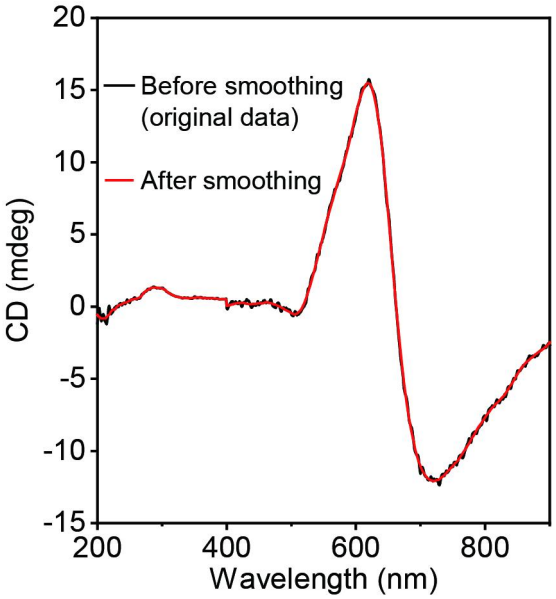

Figure S2 Typical CD spectra for AuNR–ODN_{dT22} complexes, prepared in the presence of Hg^{2+}, before and after Savitzky–Golay smoothening.

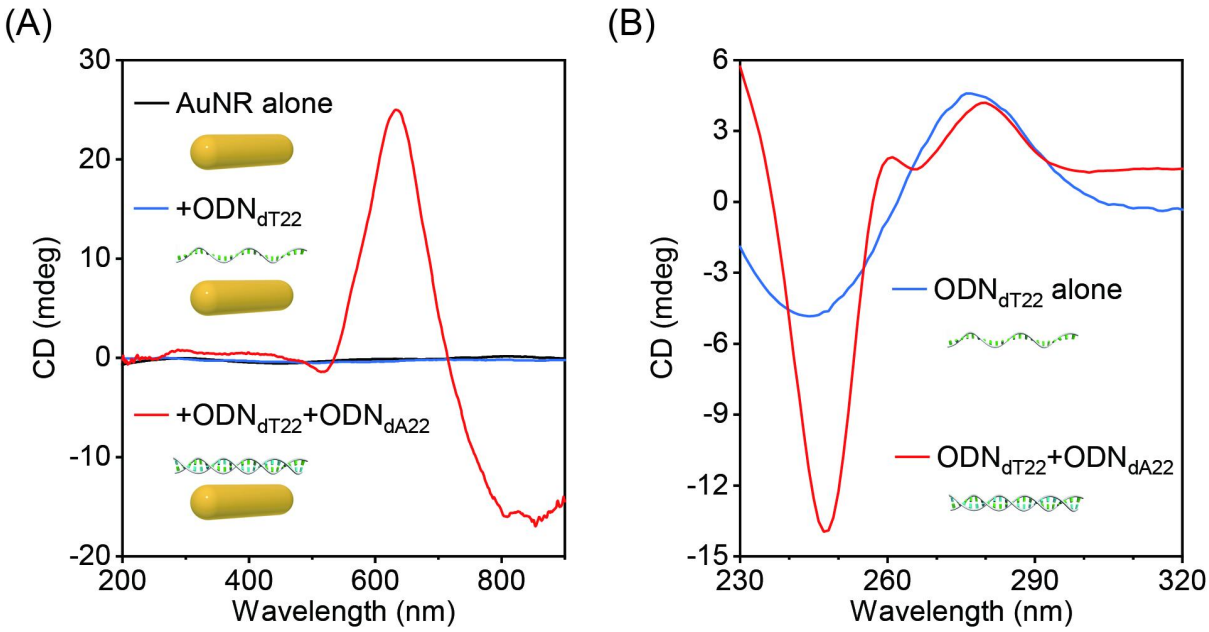

Figure S3 CD spectra for (A) AuNR alone (black line), AuNRs and 0.6 μM ODN_{dT22} complexes (blue line), AuNRs and 0.3μM ODN_{dT22} complexes with 0.3μM ODN_{dA22} (red line), (B) 20 μM ODN_{dT22} alone (blue line) and 10 μM ODN_{dT22} with 10 μM ODN_{dA22} (red line).

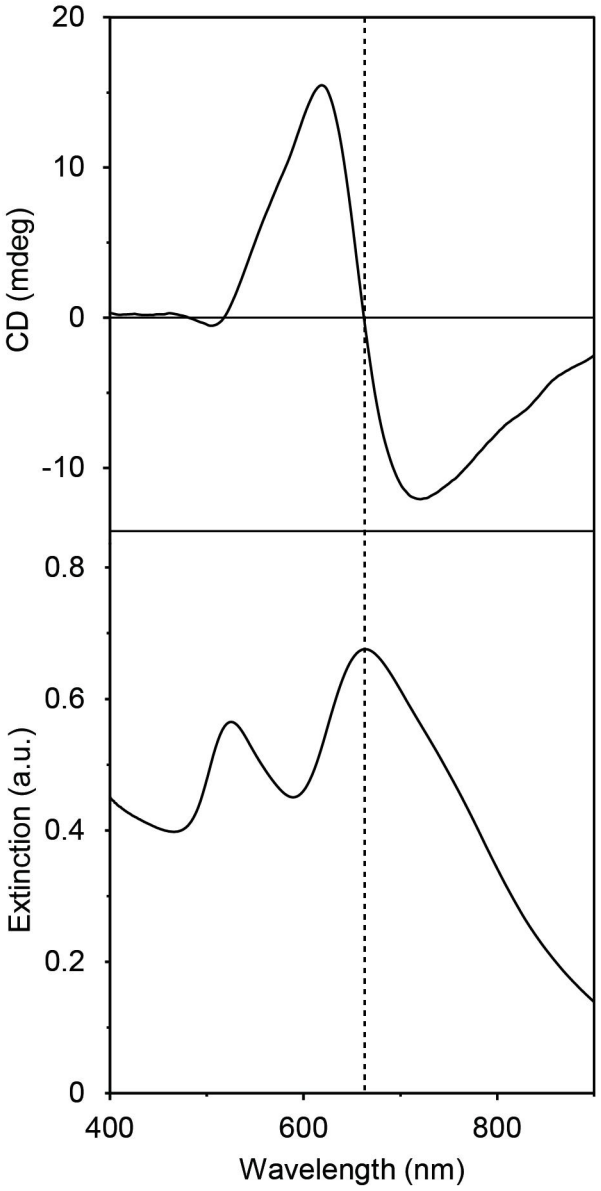

Figure S4 The CD and extinction spectra for AuNR–ODN_{dT22} complexes with Hg^{2+} (6.6 μM).

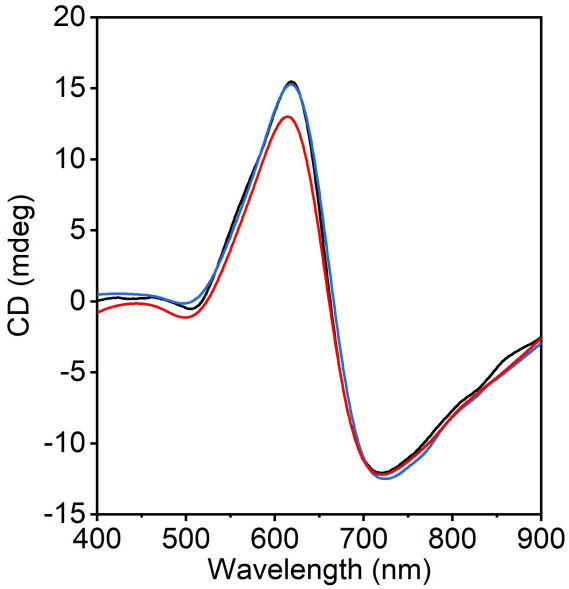

Figure S5. CD spectra for AuNR–ODNdT_{22} with 6.6 μM Hg^{2+}. To check the reproducibility, experiments were conducted on three different samples under the same conditions. The mean ΔCD intensity is 26.9±1.4 (n=3).

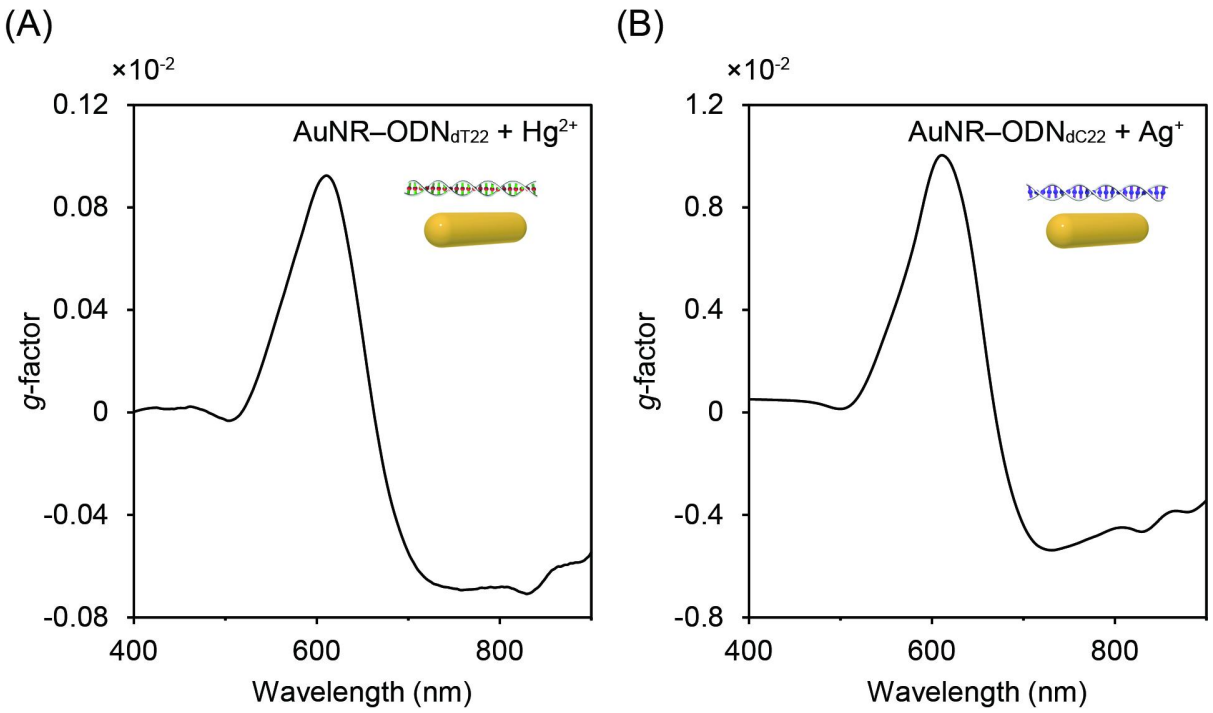

Figure S6 *g*-factor of (A) the AuNR–ODN_{dT22} complexes prepared in the presence of Hg^{2+} (6.6 μM) and (B) the AuNR–ODN_{dC22} complexes prepared in the presence of Ag^{+} (6.6 μM).

Reference

1. T. K. Sau and C. J. Murphy, *Langmuir*, 2004, **20**, 6414-6420.