Electronic Supplementary Information (ESI)

Salt Concentration-Induced Dehybridisation of DNA-Gold Nanoparticle Conjugate Assemblies for Diagnostic Applications

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Materials

The HPLC-purified DNA sequences (**SA(10A)-1**: 5′ HS-A_{10}-ATTATCACT 3′; **SA(10A)-2**: 5′ HS-A_{10}-AGTGATAAT 3′; **SA(9B)-1**: 5′ AGTGATAAT 3′; **SA(9B)-2**: 5′ ATTATCACT 3′; **Ag-1**: 5′ HS-A_{10}-ATTACCACT 3′; **Ag-2**: 5′ HS A_{10}-AGTGCTAATT 3′; **SA(15A)-1**: 5′ HS-A_{15}-AGTGATAAT 3′; **SA(15A)-2**: 5′ HS-A_{15}-ATTATCACT 3′; **SA(5A)-1**: 5′ HS-A_5-AGTGATAAT 3′; **SA(5A)-2**: 5′ HS-A_5-ATTATCACT 3′; **T20**: 5′ T_{20}-SH 3′) are purchased from Genotech (Daejeon, Republic of Korea). Quant-iT OliGreen® ss DNA Reagent (Cat.# O7582) is purchased from Invitrogen (Carlsbad, CA, USA). Dithiothreitol (DTT, Cat.# 43815), gold chloride trihydrate (Cat.# 520918), trisodium citrate dihydrate (Cat.# S4641), silver nitrate (Cat.# 204390), sodium dodecyl sulfate (SDS, Cat.# L4522) and other chemicals used for the buffer preparation are purchased from Sigma-Aldrich (Milwaukee, WI, USA). NAP-5 Sephadex columns are purchased from GE Healthcare (Piscataway, NJ, USA).

Synthesis of gold nanoparticles[1]

Prepare the aqueous solution of $HAuCl_4 \cdot 3H_2O$ (0.254 mM, 50 mL) and heat it up to boiling point while stirring. Inject a trisodium citrate solution (38.8 mM, 0.94 mL) rapidly into

the boiling solution. The colour of the solution changes from yellow to dark red. After the colour changes, allow the solution to boil for 5 min and cool to room temperature while stirring.

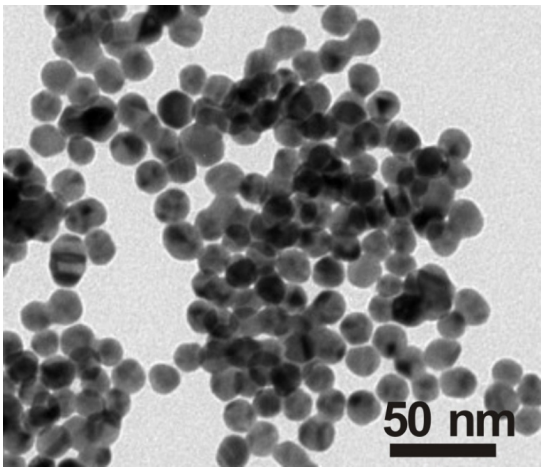

Figure 1S. A TEM image of the synthesised gold nanoparticles (15 nm in diameter)

Synthesis of DNA-gold nanoparticle conjugates (DNA-AuNPs)

Deprotect the monothiol DNA sequences by reducing the terminal disulfide group through soaking in a 0.1 M DTT solution (0.17 M phosphate buffer, pH 8.0) for 20 min before purifying the deprotected DNA using a NAP-5 column. Combine the purified DNA with the 3 nM AuNP solution (the final oligonucleotide concentration is ~ 4.7 μM). Buffer the mixed solution to 0.15 M NaCl in phosphate buffer (0.01 % SDS, pH 7.4, 10 mM phosphate) and incubate it for 24 hours at room temperature. Remove the unconjugated free DNA by repeated centrifugation, removal of the supernatant and redispersion of the DNA-AuNPs by adding the phosphate buffer (0.15M NaCl, 0.01% TWEEN 20, pH 7.4, 10 mM phosphate). Repeat this procedure three times.

Thermal melting transitions of free DNA duplexes

Prepare the solution containing two complementary sequences of DNA (**SA(9B)-1**, **SA(9B)-2**, each [DNA] = 8 μM, 0.30 M NaCl, 0.01 % TWEEN 20, pH 7.4, 10 mM phosphate). Anneal the DNA solution at 95 ^{o}C for 5 min, cool it down to room temperature, and hold for 2 hours to allow the DNA to hybridise. Heat up the hybridised DNA duplex solution from 5 to 70 ^{o}C at a rate of 1 ^{o}C/min and monitor the change in extinction at 260 nm while stirring (Agilent 8453 UV-vis spectrophotometer equipped with a Peltier temperature controller).

Salt concentration-induced melting transition of free DNA duplexes

Prepare eleven solutions, each of which contains two complementary sequences of DNA (**SA(9B)-1**, **SA(9B)-2**, each [DNA] = 8 μM, 0.01 % TWEEN 20, pH 7.4, 10 mM phosphate). Each solution contains a different concentration of salt ([NaCl] = 0, 0.045, 0.09, 0.135, 0.18, 0.225, 0.27, 0.315, 0.36, 0.405, and 0.45 M, respectively). Anneal the DNA-containing solutions at 95 ^{o}C for 5 min, cool them down to room temperature, and hold for 2 hours. After the hybridisation of DNA at each salt concentration, measure the absorbance of each solution at 260 nm at 15 ^{o}C, from which the salt concentration-induced melting transition of free DNA duplexes is obtained.

Thermal melting transitions of DNA-AuNP aggregates

Combine the two complementary DNA-AuNP solutions (each [DNA-AuNP] = 1 nM) and incubate overnight under buffer conditions (0.15 M NaCl, 10 mM phosphate, 0.01 % TWEEN 20, pH 7.4). Heat up the hybridised DNA-AuNP aggregate solution from 25 to 70 ^{o}C at a rate of 1 ^{o}C/min and monitor the change in extinction at 525 nm (Agilent 8453 UV-vis spectrophotometer equipped with a Peltier temperature controller). The thermal melting temperature (T_m) is obtained from the temperature at which the maximum of the first derivative of the melting transition occurs.

Salt concentration-induced melting transition of DNA-AuNP aggregates

The salt concentration-induced melting transitions of the DNA-AuNP aggregates are obtained by diluting the salt concentration of the aggregate solution with dilution buffer (10 mM phosphate, 0.01 % TWEEN 20, pH 7.4, no NaCl). First prepare a cuvette containing aggregates composed of two complementary DNA-AuNPs (total 1.7 pmol of DNA-AuNPs in 850 μL). Add 25 μL of the dilution buffer to the cuvette to decrease [salt], allow the mixture to equilibrate for 30 sec at a constant temperature, and measure the extinction at 525 nm, at which the dispersed DNA-AuNPs have the maximum extinction in the visible range of light (Agilent 8453 UV-vis spectrophotometer equipped with a Peltier temperature controller). To maintain a homogeneous system, stir the solution continuously with a magnetic stir bar (800 RPM). Repeat the dilution process until the melting transition takes place. The "melting concentration (C_m)" is obtained

from the concentration of NaCl at which the minimum of the first derivative of the melting transition takes place.

Detection of Ag^+ using DNA-AuNP aggregates and the cytosine-Ag^+-cytosine coordination chemistry

The two complementary DNA-AuNP conjugates (**Ag-1**, **Ag-2**) are prepared in buffer (0.15 M $NaNO_3$, 0.01 % TWEEN 20, pH 7.4, 10 mM MOPS, each [DNA-AuNP] = 2 nM, respectively) before being combined and allowed to hybridise at 4 ^oC for 24 hours. Spike 10 μL of various Ag^+ stock solutions to each aggregate sample (final [Ag^+] is 1, 3, 4, 5, 6, 8, and 10 μM, respectively). Allow the mixtures to equilibrate for 30 min while vortexing (850 RPM) at 12 ^oC. The thermal and [salt]-induced melting profiles (at 25 ^oC) of each sample are obtained as described above.

DNA loading study of DNA-AuNP conjugates used for thermal or [salt]-induced melting experiments

Prepare a batch of DNA-AuNP conjugates (**T20**) in buffer (0.15 M NaCl, 0.01 % TWEEN 20, pH 7.4, 10 mM phosphate) and divide it into three aliquots. Heat up one aliquot for thermal melting from 25 to 90 ^oC at a rate of 0.2 ^oC/min and dilute another with buffer for [salt]-induced melting by following the procedures for [salt]-induced melting described above. The other aliquot is a control and is not treated. After the simulated melting experiments, centrifuge all the DNA-AuNPs, remove the supernatants and redisperse the particles in buffer (0.15 M NaCl, 0.01 % TWEEN 20, pH 7.4, 10 mM phosphate, [DNA-AuNP] is ~ 3 nM) three times to remove the DNA strands released from the DNA-AuNPs. After measuring the concentration of DNA-AuNPs using UV-vis spectroscopy, inject 250 μL of 0.2 M DTT solution into 250 μL of the DNA-AuNP solutions to liberate the remaining DNA strands on the nanoparticles. Hold the mixtures at 40 ^oC for 30 min and spin down the particles at 13,000 RPM for 15 min. Take out 100 μL of the supernatant from each solution and transfer it to a 96-well plate. Prepare a series of free DNA solutions in the same 96-well plate for a standard curve ([DTT] = 0.1 M, [DNA] = 200, 150, 100, 50, 25, 10, 5, 1, and 0 nM, respectively). Analyse the number of DNA strands per particle by fluorescence spectroscopy using Quant-iT OliGreen® ss DNA reagent (Invitrogen)

and Gemini XPS (Molecular Device) following the manufacturer's guidelines. The experiment was in triplicate.

Spectral changes of DNA-AuNP assemblies during the [salt]-induced melting

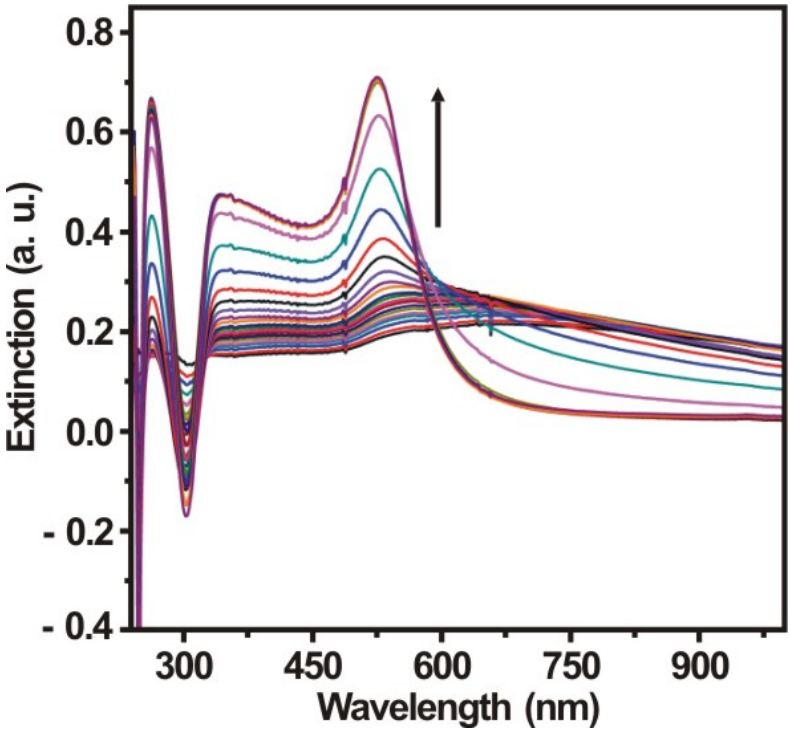

Figure 2S. Changes of the UV-vis spectra during the [salt]-induced melting of DNA-AuNP assemblies. Note that the extinction both at 525 and 260 nm increases as the salt concentration decreases. The additional decrease of the extinction by the concurrent dilution of nanoparticle concentration is mathematically corrected.

Effect of the initial particle concentration on both melting profiles

Thermal and [salt]-induced melting transitions are obtained using two complementary DNA-AuNP conjugate assemblies (**SA(10A)-1** and **SA(10A)-2**) of various concentrations in buffer (0.15 M NaCl, 0.01 % TWEEN 20, pH 7.4, 10 mM phosphate).

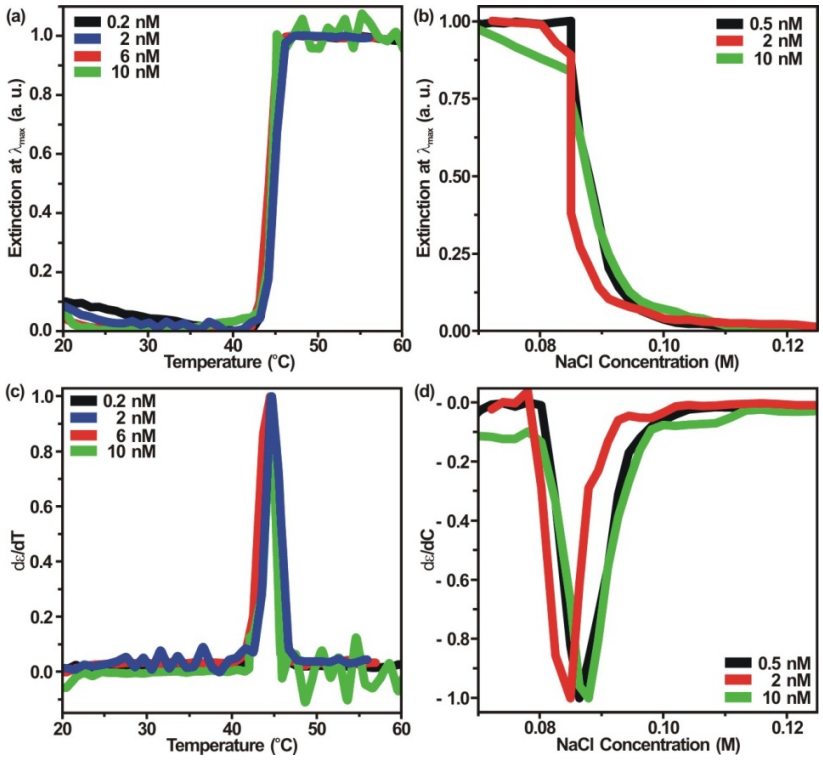

Figure 3S. Thermal and [salt]-induced dehybridisation transitions of DNA-AuNPs assemblies at various concentrations of DNA-AuNPs. (a) Thermal melting transitions of DNA-AuNP assemblies. (b) [Salt]-induced melting transitions of DNA-AuNPs assemblies at 40 ^{o}C. (c) First derivatives of the thermal melting transitions of DNA-AuNPs. (d) First derivatives of the [salt]-induced melting transitions of DNA-AuNPs. Note that extinction for DNA-AuNPs is monitored at 525 nm.

First derivatives of the [salt]-induced melting transitions at various temperatures

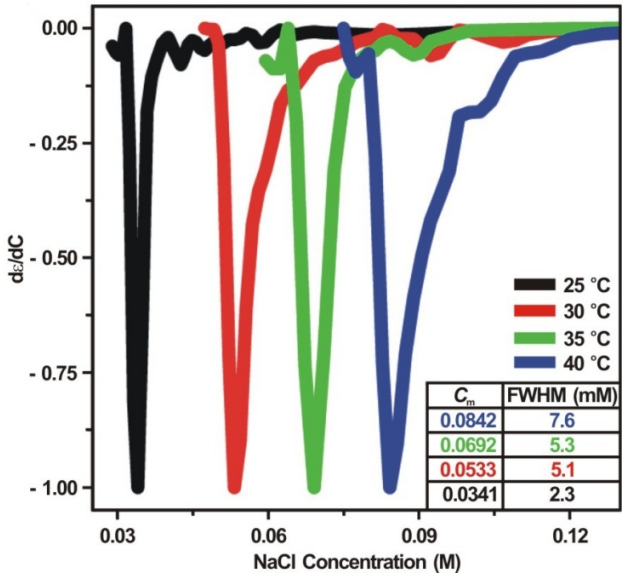

Figure 4S. First derivatives of the [salt]-induced melting transitions at various temperatures and their FWHMs. The C_m is expressed in molar concentration (M).

Reference

(1) Kumar, S.; Aaron, J.; Sokolov, K. *Nat. Protoc.* **2008**, *3*, 314-320.