ELECTRONIC SUPPLEMENTARY INFORMATION

Improving the Understanding of Oligonucleotide-Nanoparticle Conjugates Using DNA-Binding Fluorophores

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MATERIALS AND METHODS

Materials.

5'-Thioctic acid-modified oligonucleotides and the target ssDNA were purchased from ATDbio, Southampton, UK. All other chemicals were purchased from Sigma-Aldrich and used without further purification.

Nanoparticle synthesis.

Silver nanoparticles (Ag NPs) were prepared according to a modification of the method presented by Lee and Meisel.[1] An aqueous solution of $AgNO_3$ (53 mM, 2 mL) was added to Milli-Q water (100 mL) that had been heated to 40 °C. Following further heating to 97 °C, sodium citrate (43 mM, 2 mL) was added. The temperature of the reaction mixture was maintained at 97 °C for 90 minutes. The synthesis yielded spherical-like nanoparticles of ca. 50 nm diameter.[2]

Citrate stabilized gold nanoparticles of ca. 14 nm in diameter were prepared by a modified Turkevich/Frens method.[3, 4] 38.1 µmol (15 mg) of HAuCl4 trihydrate were dissolved in 150 mL of milli-Q water and heated to boiling. Subsequently, 4.5 mL of the 1 % w/w aqueous trisodium citrate solution, previously warmed to ca. 70 ˚C was added, and the mixture was refluxed for 30 minutes. The solution was then allowed to cool to room temperature under vigorous stirring. The ruby red sol was characterized by UV-Vis spectroscopy observing the typical plasmon band at 520 nm and the size distribution determined by SEM. Gold nanoparticles ca. 45 nm in diameter were prepared in a second step via a seeded growth method.[5] 37 µmol of $HAuCl_4$ trihydrate were added to 125 mL of Milli-Q water and heated to boiling. Subsequently, 5 mL of gold colloidal dispersion (ca. 14 nm sized nanoparticles acting as seeds) was added, followed by addition of 1 % w/w aqueous trisodium citrate (21.7 µmol, 638 µL). The mixture was refluxed for 30 min under vigorous stirring. To assure the stability of colloidal dispersion, 4.9 mL of 1 % w/w aqueous trisodium citrate was then added and further refluxed for 1 h. The raspberry red sol was characterized by UV-Vis spectroscopy observing the plasmon band at 534 nm. The two steps synthesis yielded gold nanoparticle of ca. 44 nm diameter.[5]

Nanoparticle functionalization and DNA assembly.

5'-Thioctic acid-modified oligonucleotides (Oligo1 and Oligo2: 10 µM, 1 ml) were added to Ag or Au colloid in a 5 ml glass vial to yield four different Ag1, Ag2, Au1 and Au2 conjugates. After 18 h, phosphate (NaH_2PO_4/ Na_2HPO_4) buffer (60 mM, pH 7.0) was added to 10 mM final concentration. NaCl (2 M) was then added at 18 h intervals, increasing the salt concentration by 0.05 M increments to a final concentration of 0.1 M. The oligonucleotide-nanoparticle conjugates were initially centrifuged at 4000 rpm for 15 minutes at which point the supernatant was removed and subsequently centrifuged at 6500 rpm for 15 minutes. The two pellets were then combined in to a single eppendorf™ tube and resuspended in 0.3 M phosphate buffered saline (PBS). This process was repeated once more and the conjugates resuspended in 0.3 M PBS. The concentrations of the conjugates were determined by UV-Vis spectroscopy using the extinction coefficients of 3.0×10^{10} M^{-1} cm^{-1} at 413 nm for Ag nanoparticles and 1.9×10^{10} M^{-1} cm^{-1} at 534 nm for Au nanoparticles. [6, 7]

Fluorescence studies described in Figure 1 and 2 were carried out as follows: ssDNA was added to Ag nanoparticle dispersions (30 pM for each set of conjugates Ag1 and Ag2. i.e. in the Ag1-Ag2 mixture the final nanoparticle concentration is 30 + 30 = 60 pM) in PBS 0.3 M up to a final concentration of 5 nM and left overnight for a full hybridization. Afterwards, SG solution was added at the constant ratio of dbpr = 1 (i.e in the

case of Ag1-Ag2 mixtures, the final SG concentration was twice as that of individual Ag1 or Ag2 dispersion). After 1 hour, the absolute intensity (Figure 1) and then the T_m (Figure 2B) were measured. A control experiment with a non-complementary target (5 nM) was similarly carried out on a Ag1-Ag2 mixture (30 pM for each set of conjugates) to assess the specificity of the DNA-mediated aggregation.

In the case of T_m measurements for 15 nM ssDNA (Figure 2B, blue line), the Ag1-Ag2 mixture in PBS 0.3 M was initially left to assemble overnight by addition of 5 nM (final concentration) and then a second addition of ssDNA corresponding to 10 nM was performed (i.e., the final ssDNA concentration in the sample was then 5+10=15 nM).

The corresponding fluorescence studies in the absence of nanoparticles (Figure 2A) were carried out in the same way but replacing the Ag1 and Ag2 conjugates with free Oligo1 and Oligo2 strands in equimolar concentrations with respect to the target ssDNA (5 nM).

In the case of Au conjugates, the fluorescent measurements for the Au1-Au2 mixture were carried out as for Ag nanoparticles but with reduced ssDNA concentration (3 nM) since higher target concentrations hampered, on the contrary, an extended nanoparticle aggregation.

The target concentration, for both Ag1-Ag2 and Au1-Au2 probes, was roughly optimized to induce extensive formation of aggregates but at the same time to guarantee the stability of the clusters in the colloidal solution over large period of time (at least several hours). This was intended, on the one hand, to increase the sensitivity of fluorescence measurements in revealing enhancement/quenching effect in single particle systems vs. cluster systems (Fig. 1), and improve the accuracy of the melting measurements in Fig. 2; and, on the other hand, to avoid unreliable results caused by a significant cluster deposition during the experimental time scale.

Fluorescence hybridization study shown in Figure 3 was performed on Ag1 conjugates as follows. Different aliquots of ssDNA+SG solution (dbpr = 10) were added to Ag1 dispersions (10 pM) and the left overnight. The nanoparticles were then collected by centrifugation and washed two times with PBS 0.3 M to remove unbound target and SG molecules from the solution. The nanoparticles were finally redispersed in equal volumes of PBS 0.3 M and the fluorescence intensity was measured. In this study, a large excess of SG was used to assure the full dye loading onto the double-helix for each investigated sample (i.e. to assure that increase in fluorescence intensity is directly related to the extent of the hybridization event).

UV-Vis absorption and Fluorescence measurements.

UV-Vis analysis was performed on a Varian Cary Bio 300 spectrometer. Extinction duplex melting experiments of oligonucleotide-modified Ag and Au nanoparticles were recorded at 413 nm and 534 nm respectively, and the temperature was programmed to a heat/cool rate of 0.5 °C/minute. Fluorescence measurements were monitored using a Stratagene MX3000P QPCR and a FAM filter set (Excitation = 492 nm, Emission = 516 nm).

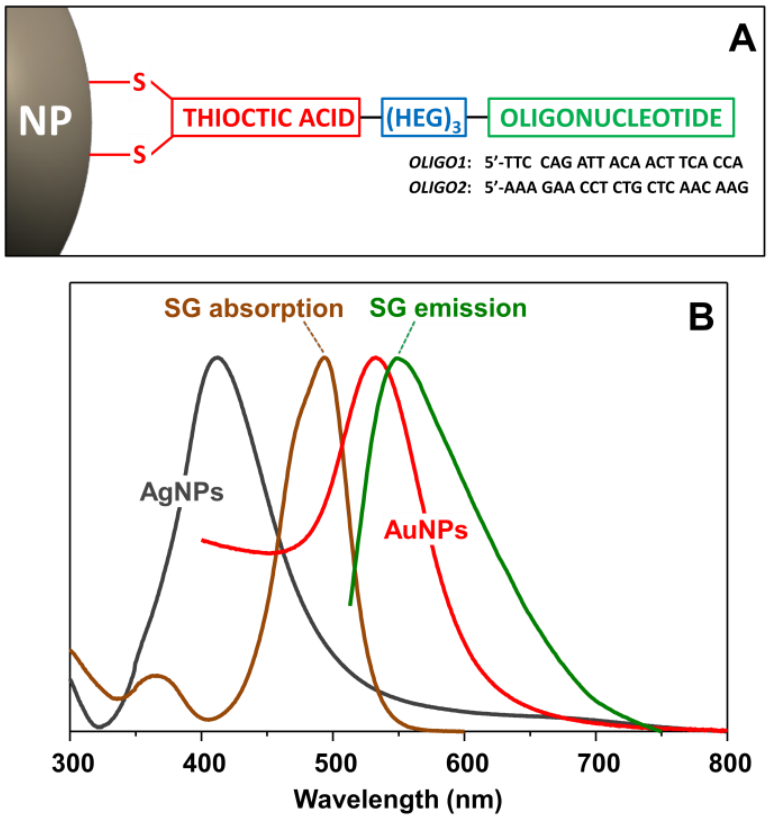

Figure S1. [A] Schematic description of the ONPC system. [B] Normalized extinction spectra of Ag citrate and Au citrate colloids, together with the normalized absorption and emission (λ_{ex} = 495 nm) spectra of SG in aqueous solution.

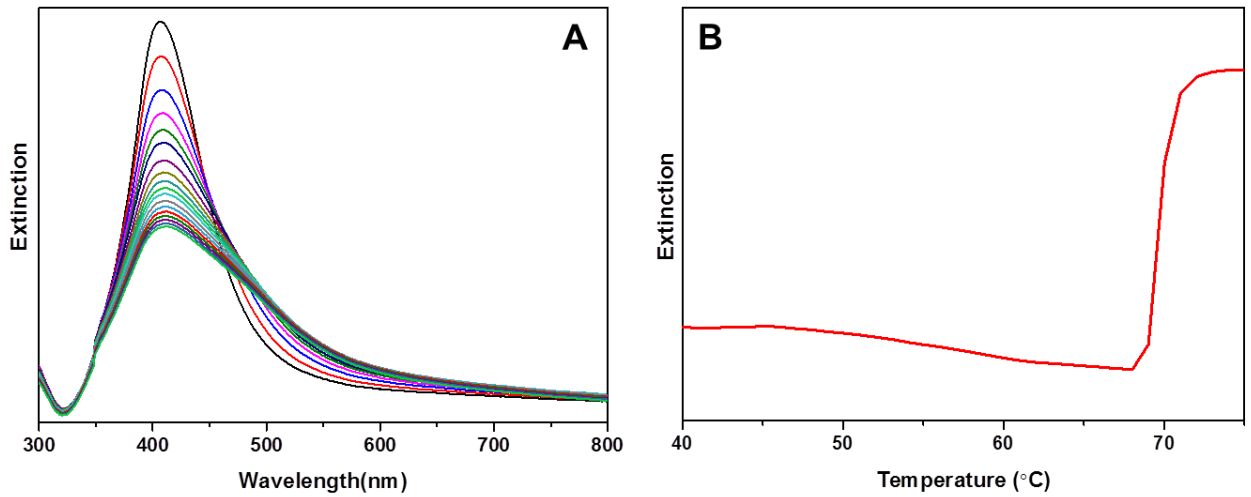

Figure S2. [A] Extinction spectra of the Ag1-Ag2 mixture ([Ag1]= [Ag2]=30pM) upon addition of target ssDNA (5nM). [B] Extinction duplex melting transitions of Ag1-Ag2 mixture (target ssDNA = 5 nM). Melting experiments were recorded at 412 nm and at a heat/cool rate of 0.5 °C/minute. Melting transitions take place at 71.0 °C.

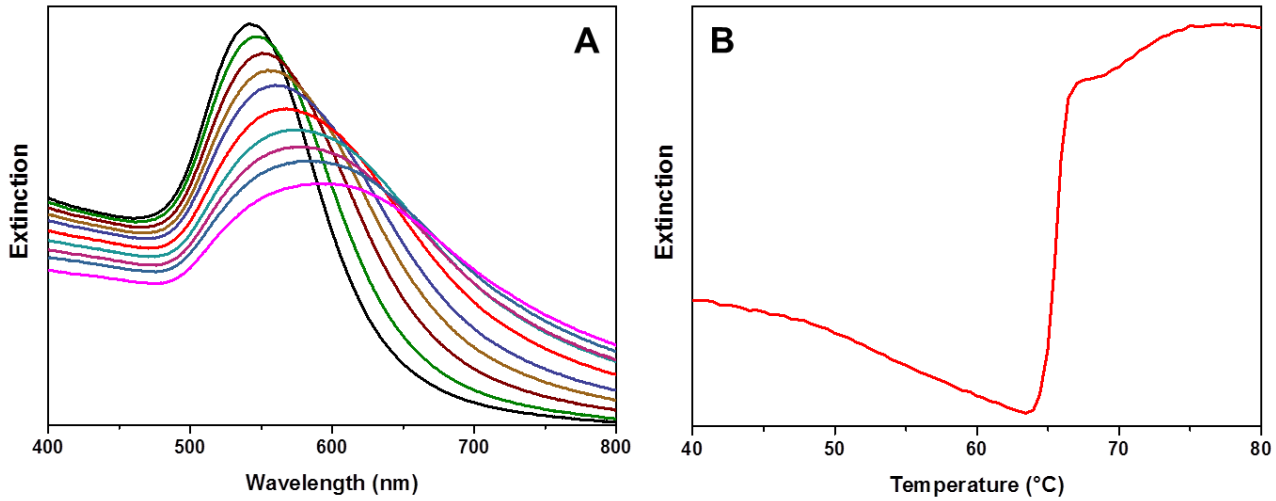

Figure S3. [A] Extinction spectra of the Au1-Au2 mixture ([Au1]= [Au2]=30pM) upon addition of target ssDNA (3 nM). [B] Extinction duplex melting transitions of Au1Au2 probes (target ssDNA = 3 nM). Melting experiments were recorded at 530 nm and at a heat/cool rate of 0.5 °C/minute. Melting transition takes place at 65.8 °C.

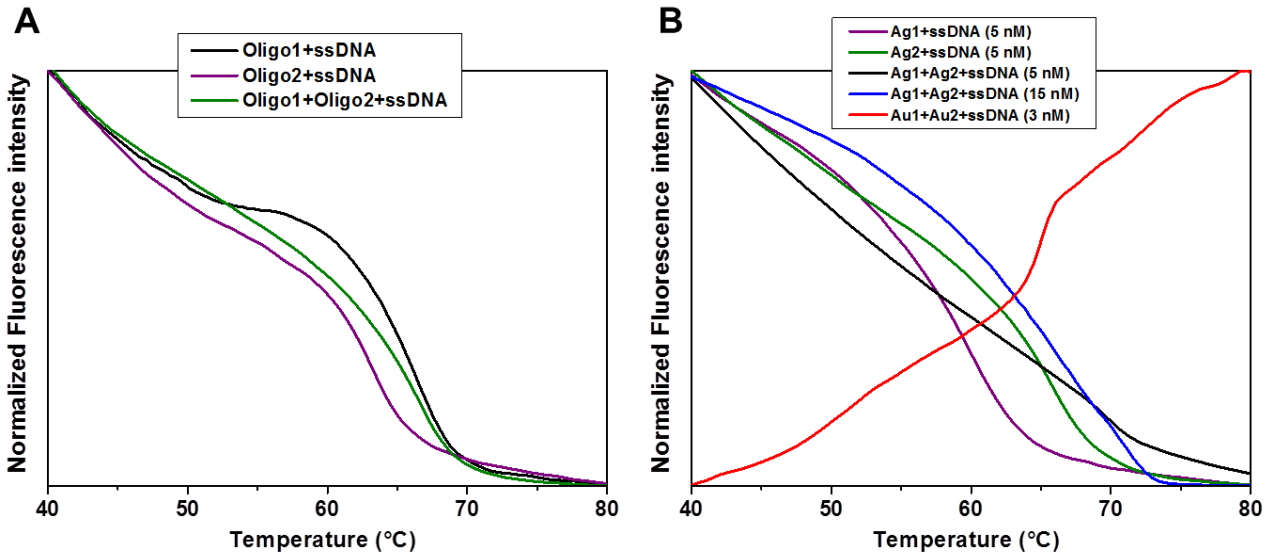

Figure S4. Temperature dependence of the normalized fluorescence intensity of: [A] free Oligo1, Oligo2 and Oligo1-Oligo2 with ssDNA in solution (Oligo1=Oligo2=ssDNA=5 nM), purple, green and black line, respectively. SG (dbpr = 1.0); [B] Ag1, Ag2 and Ag1-Ag2 probes for target ssDNA = 5 nM, purple line, green line and black line, respectively. Ag1-Ag2 probes for target ssDNA = 15 nM, blue line. Au1-Au2 probes for target ssDNA = 3 nM, red line. SG (dbpr = 1.0).

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