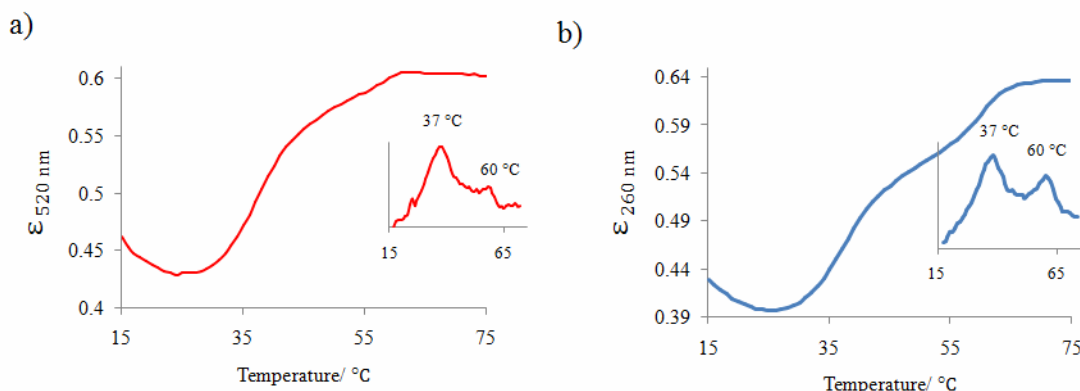


LNA Functionalized Gold Nanoparticles as Probes for Double Stranded DNA through Triplex Formation

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UV-Vis triplex melting curves



UV-Vis triplex melting curves at a) 520 nm b) 260 nm. First derivatives and melting temperatures shown in insets.

Experimental Section

The gold nanoparticles were prepared via citrate reduction of HAuCl_4 . The concentration of the gold nanoparticles was increased by centrifugation and resuspension to obtain a final concentration of 37.5 nM. HPLC thiol-capped LNA/DNA chimeras with a 20 adenine linker between thiol group and DNA sequence (Eurogentec, Belgium) were added to the aqueous nanoparticle suspension (final concentration of oligonucleotide 3 μM). After 24 hours, the suspension was brought to 10 mM phosphate ($\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$) by addition of 60 mM pH 7 buffer. Following a further 24 hours, the suspension was brought to 0.1 M NaCl by several additions of 2 M NaCl solution over 48 hours. The nanoparticles were centrifuged to remove excess oligonucleotide and were resuspended in PBS. The final concentration of the LNA/DNA gold conjugates, obtained using UV-Vis spectroscopy, was 10-100 nM.

The nanoparticle assemblies were prepared by mixing the target double stranded DNA (100 nM) with the 2 oligonucleotide probes (1 nM) in 0.5 M PBS buffer, pH 7 at room temperature. The oligonucleotide sequences used are shown in figure 1. Unmodified oligonucleotides were hybridised in a 1:1 ratio (100 nM) in 0.5 M PBS. The melting experiments were conducted by monitoring the change in absorbance at 260 nm and 520 nm, using a Varian CaryWin UV-Vis spectrometer.