LNA functionalized gold nanoparticles as probes for double stranded DNA through triplex formation†

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Nanoparticle modified LNA probes have been used for colorimetric identification of double stranded DNA *via* parallel triplex formation, eliminating the need for prior denaturation of the target duplex.

Over the last decade there has been a significant amount of research dedicated to the properties and applications of DNA functionalized gold nanoparticles. DNA in the form of synthetic oligonucleotides has been conjugated to the surface of gold nanoparticles and used in a variety of different studies including hybridizations for specific sequences,[1,2] assembly of 3-dimensional aggregates,[3,4] immobilization onto surfaces and sensing of protein species *via* DNA protein interactions.[5] The protein interactions aside, the majority of these functionalized gold nanoparticles have been used to hybridize to single stranded DNA sequences using Watson Crick base pairing. This has resulted in excellent sensitivity and sequence specificity due to the discriminatory effect of the gold nanoparticles on the ability of the oligonucleotide sequences to hybridise the DNA target. Sharp melting transitions over 2–3 °C have been obtained and in some cases the combination of gold nanoparticles hybridizing to specific DNA sequences coupled with a highly sensitive silver reduction has resulted in detection of DNA without the use of the polymerase chain reaction.[6]

DNA in its natural state is double stranded and as such it is attractive to try and develop methodologies that can detect double stranded DNA and hence do not require denaturation into single strands as is required in most commonly used approaches. Triplex forming oligonucleotides (TFOs) have been reported many times in the literature;[7–9] however, they have never been reported as conjugated to gold nanoparticles for use in the detection of a specific double stranded DNA sequence. Previous work has used gold nanoparticles conjugated to oligonucleotides to assess the behaviour and stability of DNA triple helixes but used as a self complementary loop sequence which was attached to one gold nanoparticle and the triplex forming oligonucleotide attached to another gold nanoparticle.[10] In a similar study, Jung *et al.* reported the use of DNA functionalized gold nanoparticles to examine the proton fuelled reversible assembly of triplex formation.[11] Both of these studies used the interaction of the gold nanoparticles and the resulting change in plasmon resonance when the nanoparticles are pulled closer together on triplex formation. These approaches are not useful for the detection of a specific duplex since they rely on one of the target DNA sequences being modified with gold nanoparticles. In addition, these studies used unmodified DNA and as such required an acidic pH to form the triple helixes through the protonation of the deoxycytidine residues. An alternative to using acidic conditions is to replace some of the DNA nucleosides with locked nucleic acids (LNA). Investigations to find the optimum number of LNA nucleotide modifications per oligonucleotide have shown that one modification every 2–3 bases to be ideal.[12] This ratio of LNA to DNA nucleotides yields the greatest thermal stabilities, whereas fully modified LNA oligonucleotides inhibit triplex formation.[13] Further to this, the 5 position of deoxycytidine can be methylated to increase the pK_a and thus promote the protonation potential at neutral pH. This allows the formation of a triple helix at room temperature under normal physiological conditions.[12–14]

In a recent study, we have demonstrated the ability of LNA functionalized gold nanoparticles to provide excellent sequence selectivity compared to DNA functionalized gold nanoparticles for duplex formation.[15] Here, we report the advancement of that investigation by using the LNA functionalized gold nanoparticles in a split probe arrangement to detect a sequence of duplex DNA through triple helix formation. The LNA functionalized gold nanoparticles were synthesized as previously reported[15] and used 20 adenine spacers onto the surface of the gold nanoparticles, which were calculated to be 13 nm in diameter using TEM measurements. Seferos *et al.* have calculated the number of LNA oligonucleotides for 13 nm gold nanoparticles to be approximately 205 strands per particle.[16] The target chosen for detection was that of the mouse nitric oxide synthase (NOS) gene, where a region of purine residues was located. It should be noted that triplex formation is optimal when there is a polypurine target; however, recent studies have indicated that specially designed base analogues targeted directly against runs of mixed purines and pyrimidines will allow triplex formation from a greater range of sequences,[17] however, as yet these modified nucleosides are not commercially available.

A 14 base target was chosen from the mouse gene and two 7-mer LNA modified probes were synthesized as shown in Fig. 1. Three LNA bases were used per probe and each was synthesized with an alkyl thiol, which was used to immobilise the LNA probes onto the gold nanoparticles. All the deoxycytidine residues were methylated at the 5 position

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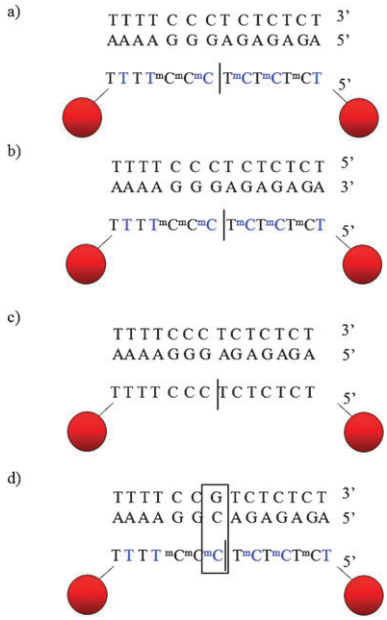

Fig. 1 (a) Nanoparticle-modified parallel LNA oligonucleotide sequences used for triplex formation with double stranded DNA; (b) and (c) control samples incorporating nanoparticle-modified anti-parallel LNA sequences and parallel DNA sequences, respectively; (d) parallel LNA sequences with mismatch target DNA. The boxed region indicates the single base pair mismatch. The linkage between gold nanoparticles and DNA–LNA sequence represents 20 adenine bases. Blue letters denote LNA bases. ^{m}C represents 5-methyldeoxycytosine.

including those of the DNA to increase protonation and hence triplex formation at physiological pH. Fig. 1a shows the duplex targeted by the triplex forming split probes to form the parallel triplex through the Hoogsteen base pairing bringing the gold nanoparticles into close proximity. Fig. 1b represents the LNA probes mixed with the reverse double stranded DNA sequence. This combination of DNA target sequence and anti-parallel probe sequence is the same as would be used for DNA sequence detection where denaturation is required. Fig. 1c shows analogous DNA nanoparticle probes of parallel sequence to the target duplex.

The visual colour change of the nanoparticles and the corresponding extinction spectra on addition of the double stranded DNA is shown in Fig. 2. DNA addition takes place at room temperature and the samples are not heated and cooled to form the resultant hybrids. Addition of the double stranded DNA to the triplex forming LNA probes clearly shows aggregation of the nanoparticles, illustrated by a red shift in the plasmon resonance peak (Fig. 2a), accompanied by a dramatic colour change from red to purple (Fig. 2b). Melting curves conducted at 520 nm and 260 nm wavelengths (shown in the ESI†) illustrate a melting temperature of 37 °C, which is consistent with the observation of hybridisation-induced aggregation of the nanoparticles at room temperature. Use of analogous DNA probes to hybridise to the target duplex did not result in any nanoparticle aggregation, indicating that the LNA and 5-methyldeoxycytidine modifications are an essential requirement for triplex formation under the conditions used. The LNA probes were also added to a duplex of reverse sequence (Fig. 1b). Probe hybridisation and subsequent

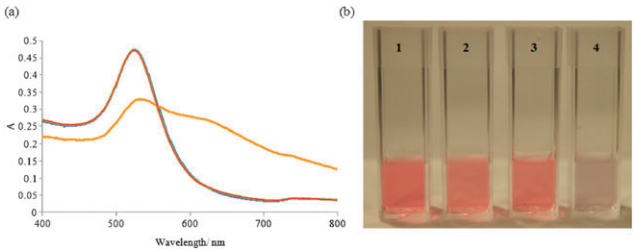

Fig. 2 Extinction spectra (a) and corresponding photograph of nanoparticle probes obtained 1 hour after sample preparation (b). Red line/cuvette 1: LNA probes before double stranded DNA addition; blue line/cuvette 2: DNA probes after double stranded DNA addition (Fig. 1c); green line/cuvette 3: LNA probes after addition of reverse sequence double stranded DNA (Fig. 1b); orange line/cuvette 4: LNA probes after double stranded DNA addition (Fig. 1a).

aggregation of the nanoparticles would only be anticipated if the probes formed an anti-parallel triplex or were able to invade the complementary DNA strands and hybridise to the target strand through Watson Crick base pairing. Nanoparticle aggregation from the sequences shown in Fig. 1b was observed but only after a time period of 24 hours had lapsed. The time limitation associated with using anti-parallel probes emphasises the advantage of using the parallel LNA TFOs for targeting double stranded DNA. Repetition of the experiment using analogous DNA nanoparticle probes (Fig. 1c) did not result in nanoparticle aggregation indicating the hybridisation properties observed are unique to the LNA nanoparticle probes under the conditions used.

The sequence selectivity of the TFO LNA probes was investigated by using target double stranded DNA that incorporated a single base pair mismatch (Fig. 1d). The mismatch target DNA was added at room temperature and the extinction spectrum was monitored, as shown in Fig. 3.

Addition of the complementary target results in complete aggregation of the nanoparticles within 30 minutes, whereas addition of the mismatch target has a negligible effect on the plasmon resonance of the nanoparticle probes within the same time frame. After an hour at room temperature, the mismatch target can clearly be differentiated from the complementary target due to the large colorimetric difference between the two samples (Fig. 4).

In summary the ability of split LNA probes conjugated to nanoparticles to selectively form a triplex and hence target a region of double stranded DNA without denaturation has

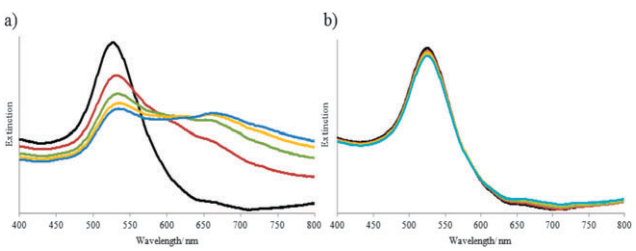

Fig. 3 Extinction spectra of (a) complementary triplex and (b) mismatch triplex monitored over 30 minutes at room temperature. Black line: 0 minutes; red: 6 minutes; green: 12 minutes; orange: 18 minutes; blue: 24 minutes.

Fig. 4 Photograph of nanoparticle probes obtained 1 hour after addition of double stranded DNA. Left cuvette: complementary target; right cuvette: target incorporating a single base pair mismatch.

been demonstrated. This is the first such demonstration to our knowledge and indicates that there is no need to denature double stranded DNA if targeting a polypurine region of the duplex. Use of specialist modified nucleosides will allow this approach to be used to target almost any region of double-stranded DNA and opens up the possibility of a very simple colorimetric indicator of the presence of sufficient quantities of double-stranded DNA in a very sequence specific manner.

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