Single Base Mismatch Determination using DNA - Silver Nanoparticle Conjugates

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ABSTRACT

DNA - gold nanoparticle conjugates were first synthesized in 1996 and have been used as bioanalytical tools for the detection of DNA and proteins as well as a new form of molecular building block. However, most of this research is concerned with gold, with little focus upon other types of nanoparticles. The research presented here shows the analogous DNA - silver nanoparticle conjugates have similar characteristics and coupled with an extinction coefficient 100× larger makes more sensitive detection possible. We report the first use of DNA - silver nanoparticle conjugates for single base mismatch determination in a DNA sandwich assay. The hybridization of the conjugates was monitored via a variety of methods. Furthermore, a completely new form of conjugate has been synthesized which has a Raman active species on the surface of the nanoparticle. Surface Enhanced Resonance Raman Scattering (SERRS) has been used to detect the sequence specific hybridization of these DNA – Raman dye – nanoparticle (DDN) conjugates.

Keywords: DNA – Nanoparticle conjugates, silver, SERRS

1 INTRODUCTION

Easy, ultra-low concentration detection of oligonucleotides has been the aim of a great deal of research for decades and to that end DNA has been modified with a large range of functional moieties including fluorescent markers[1], proteins[2], radioactive elements[3] and chemillumenescent compounds[4] and with each step the concentration boundary is pushed further. Modification of oligonucleotides with gold nanoparticles was first reported ten years ago [5][6] and opened up several new avenues of research as they can be used as a novel nanostructured building material but the DNA – gold nanoparticle conjugate can also be used to colorimetrically detect low concentrations of a target oligonucleotide, differentiating between full complementarity and a single nucleotide polymorphism visually when used in a sandwich assay format [7]. This is made possible because the nanoparticle alters the melting characteristics of the DNA duplex making the change from double stranded to single stranded much sharper when compared to unmodified oligonucleotides. The sharper transition has been determined to be a cooperative melting effect that results from multiple hybridisation events occurring between conjugates and a decrease in the melting temperature as DNA stands melt due to a concomitant reduction local salt concentration. By modifying the DNA means the melting temperature is not only dependent upon target oligonucleotide concentration and the ionic strength of the buffer medium but the size of the conjugated nanoparticle, the distance between the nanoparticles during hybridisation and the density of the oligonucleotide on the surface of the nanoparticle [8]. Also, the transition can be monitored at multiple wavelengths; 260nm (oligonucleotide maxima), 520nm (unaggregated gold nanoparticle surface plasmon maxima) and 650nm (aggregated gold nanoparticle surface plasmon maxima) via conventional spectroscopy. Whilst the novel aspects of the nanoparticle label have lend themselves to a wide variety of array – based detection methods including use of a conventional flatbed scanner, Raman spectroscopy[9] and conductivity measurements[10]. All these techniques use a silver enhancement amplification methodology to coat the conjugate with silver metal and increase the visibility of the label or the generated signal. In a similar method to oligonucleotide analysis colorimetric protein detection has been achieved by conjugation of an aptamer sequence to the nanoparticle [11].

However, almost all the research focus has been concerned with using gold nanoparticles with very little work on other noble metal nanoparticles such as silver [12]. This is due to the synthesis method making it difficult to attain repeatable size and optical characteristics. What work has been done utilises direct conjugate – conjugate hybridisation or hybridisation to a functionalised surface.

A benefit of using silver nanoparticles is that they open the door to other detection techniques with out the need for an amplification step. As previously stated Raman spectroscopy, namely surface enhanced Raman scattering (SERS) has been used in conjunction with DNA – gold nanoparticle conjugates. That technique used a dye modified oligonucleotide and silver amplification to attain a signal. With silver nanoparticles a dye molecules can be

complexed to the surface along with alkylthiol oligonucleotides, creating a dual – functionalised Raman – active DNA – silver nanoparticle conjugate.

2 RESULTS

2.1 Functionalisation of Nanoparticles

By using conventional solid phase phosphoramidite chemistry a thiol functional group was added at either the 3' or 5' position on a DNA strand. This modification was used to complex the single stranded oligonucleotide to the nanoparticle via the well documented thiol attachment chemistry. In this form the conjugates are sensitive to changes in conditions so a hybridisation buffer of phosphate and sodium chloride solution was added over the course of several days.

Sequences used:

Probe 1: 5'thiol 20A-TCTCAACTCGTA
Probe 2: 5'thiol 20ACGCATTCAGGAT
Complementary: TACGAGTTGAGAATCCTGAATGCG
Mismatch head: TACGAGTTGAG***C***ATCCTGAATGCG
Mismatch tail: ***C***ACGAGTTGAGAATCCTGAATGCG
Nonsense: GCTGGAACAGTCCTTAGCCGAGCT

2.2 Hybridization of DNA – Silver Nanoparticle Conjugates

Figure 1 Visible image of hybridizing DNA – silver nanoparticle conjugates to a fully complementary target (left) and a non complementary strand (right)

Figure 1 shows the visual change that occurs when DNA – silver nanoparticle conjugates are hybridised. The cuvette on the left had a fully complementary target oligonucleotide whilst the cuvette on the right has a nonsense sequence. This was experiment was performed with 30 attomoles of conjugate and 3 picomoles of target oligonucleotide in 1ml and demonstrates the sequence-specific aggregation and surface plasmon change of the nanoparticle conjugates.

This change can also be monitored using UV-visible spectroscopy (Figure 2) by taking repeated full spectrum scans over 3 hours the change from the unaggregated/non-hybridised λmax of 407nm to the aggregated/hybridised λmax of 540nm is observed.

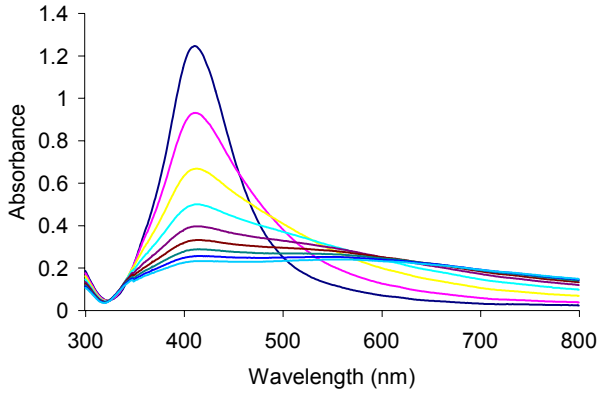

Figure 2 UV – vis spectra of hybridizing DNA – silver nanoparticle conjugates

2.3 Single Base Mismatch Determination using DNA – Silver Nanoparticle Conjugates

Figure 3 shows the difference in melting curves and melting temperature (T_m) between a fully complementary sequence and a single base mismatch. Comparing this to the melting curve of non-modified DNA it is clear that the change in absorbance is larger and the change from double stranded (aggregated) to single stranded (unaggregated) is much sharper. What the graph does show is the different wavelength necessary for the analysis of DNA – silver nanoparticle conjugates. Rather than monitoring at 520nm as you would for DNA – gold nanoparticle conjugates, the change is seen at 407nm.

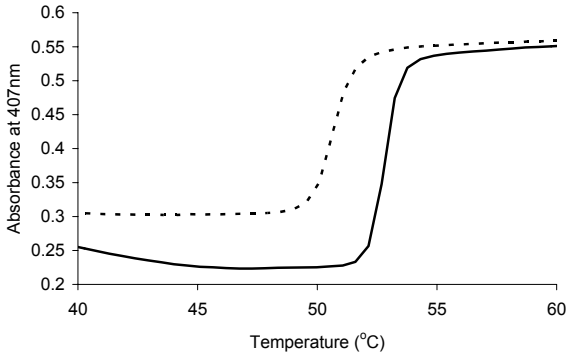

Figure 3 Melting curves attained for a fully complementary (filled line) and single base mismatch (broken line) target oligonucleotides

This difference can also be measured colorimetrically via spotting onto a RP TLC plate. Figure 4 shows the

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results of an assay of fully complementary, single mismatch and nonsense strands. Samples are taken every degree through the melting temperature and this is illustrated by the color change from grey (double stranded/aggregated) to green (single stranded/not aggregated). The single base mismatch changes at a lower temperature compared to the fully complementary strand whilst the nonsense shows no change from green.

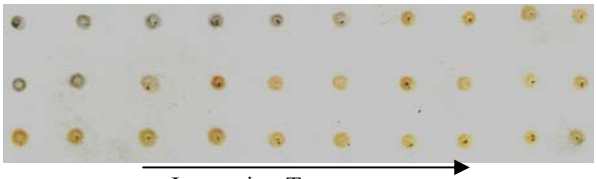

Figure 4 Visual image of a reverse phase thin layer chromatography plate spotted with hybridizing DNA – silver nanoparticle conjugates.
(Top = complementary, middle = mismatch, bottom = non complementary)

2.4 Synthesis of DNA – Raman dye – Nanoparticle Conjugates

Combining the synthesis of a DNA nanoparticle conjugate with the incorporation of a Raman active species has not been attempted elsewhere. The synthesis procedure of DNA – dye – silver nanoparticle (DDN) conjugates is identical to that for DNA – silver nanoparticle conjugates, apart from the addition of the dye to the nanoparticles prior to the addition of the thiol modified DNA. The dye used is 5-Dimethoxy-4-(6'azobenzotriazolyl)phenol (Figure 5).

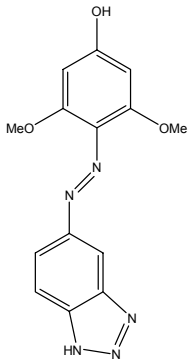

Figure 5 5-Dimethoxy-4-(6'azobenzotriazolyl)phenol

2.5 Hybridization and Single Base Mismatch Determination of DNA – Raman dye – Nanoparticle Conjugates

In theory SERRS can be used to detect the hybridization of DDN conjugates as when in their single stranded state in the presence of a nonsense strand the particles will be unaggregated and the SERRS signal will be small (or non-existent). When a complementary strand is added the conjugates will aggregate and the surface plasmon will shift giving a SERRS spectra. Figure 6 shows the change in SERRS spectra of a DDN conjugate hybridizing to a complementary strand.

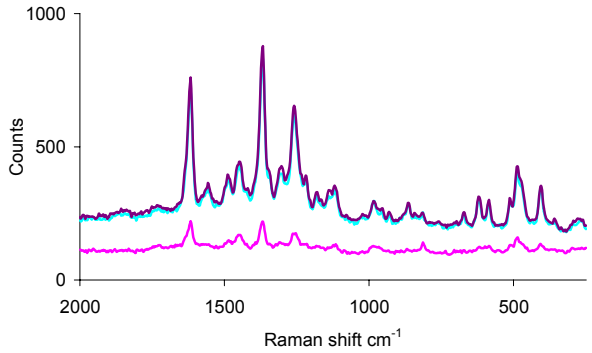

Figure 6 SERRS spectra of DDN conjugates hybridizing to a complementary target illustrating the increase in intensity

When attempting to use DDN conjugates to determine a single base mismatch it became clear through experimental work that this could not be done through conventional melting means. However by altering the placement of the mismatch in the target oligonucleotide (section 2.1) it was possible (Figure 7).

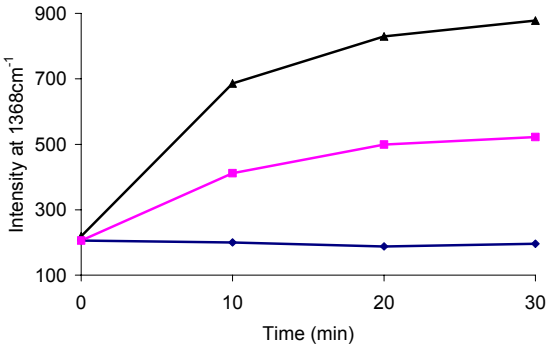

Figure 7 The change in major peak intensity of DDN conjugates when hybridizing to a complementary (top), mismatch (middle), non complementary (bottom) target.

3 CONCLUSION

Working towards the ultimate goal of a nanoscale DNA biosensor a number of the aims have been achieved. Functionalisation of both gold and silver nanoparticles with thiol modified DNA has been done successfully and repeatably to give stable DNA – nanoparticle conjugates that can be used for further analyses. The sequence specific hybridization of the DNA – nanoparticle conjugates in a sandwich assay has also been achieved and monitored using UV spectroscopy and colorimetry and been used to determine single base mismatches in a target

oligonucleotide. Finally by using a benzotriazole functionalized dye, hybridization of conjugates to a target has been used to generate a SERRS spectrum. These advances show that DNA – silver nanoparticles have great promise for use in the future as an analytical tool for DNA detection and sequence determination.

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