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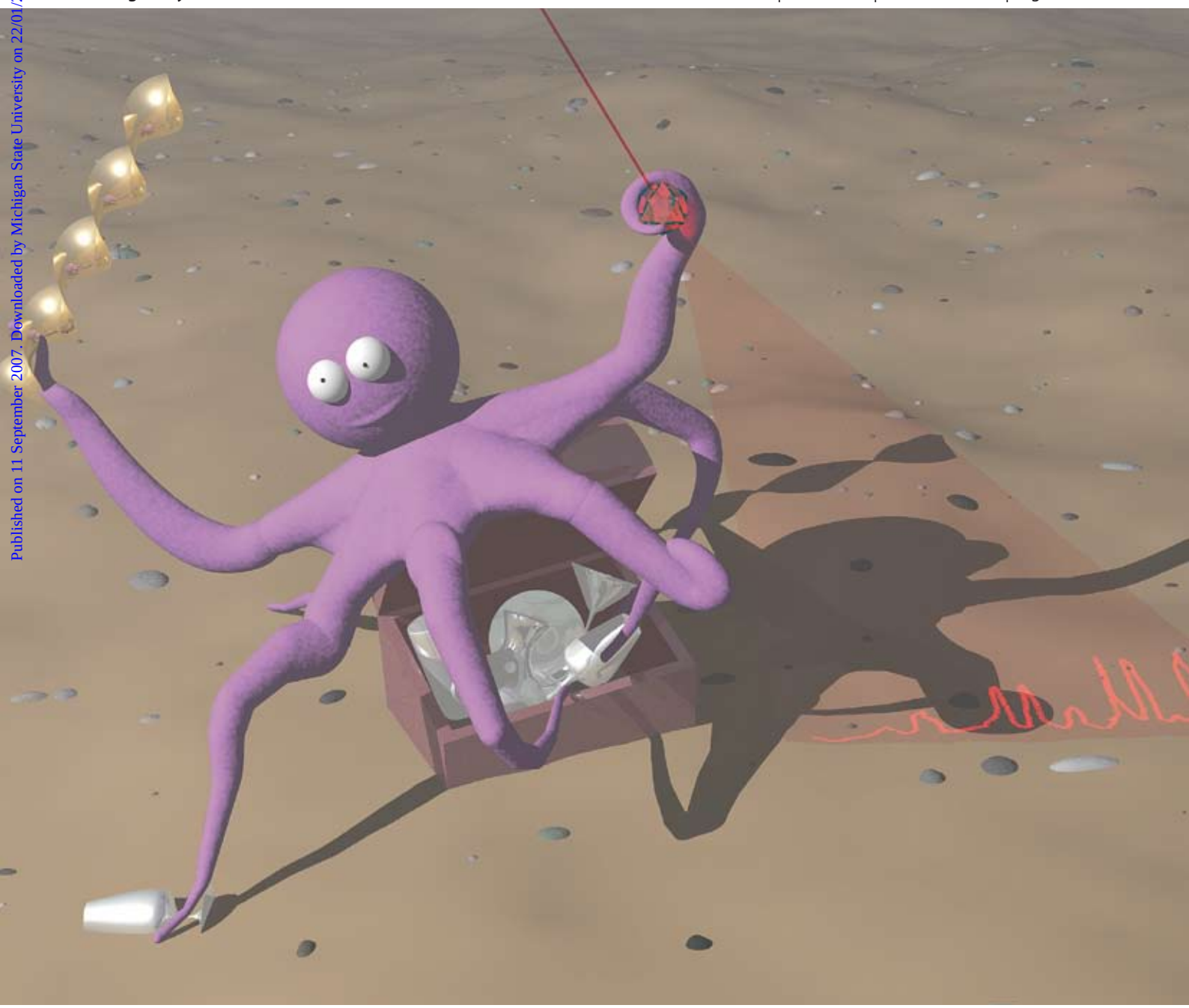

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Evaluation of the number of modified bases required for quantitative SERRS from labelled DNA

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Evaluation of the number of modified bases required for quantitative SERRS from labelled DNA

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The optimisation of the modification of DNA to facilitate quantitative detection by surface enhanced resonance Raman scattering (SERRS) detection is reported.

A number of studies have investigated the quantitative nature of the detection of labelled oligonucleotides using surface enhanced resonance Raman scattering (SERRS).[1–3] Previous studies have shown that the nature of the dye label has a great influence on the SERRS response obtained.[4] This is due to the requirement of adsorption of a coloured molecule onto a roughened metal surface to provide the maximum surface enhancement and also optimal resonance contribution from the overlap of the electronic transitions of the dye and the excitation frequency used in the scattering experiment. In almost all of the previous studies into the SERRS detection of labelled oligonucleotides silver nanoparticles have been used. Silver nanoparticles are traditionally prepared by the reduction of a silver salt, normally silver nitrate, using a variety of different reducing agents such as sodium borohydride, or sodium citrate.[5–7] Nanoparticles produced in this way typically have a zeta potential of around −45 mV and as such are highly negatively charged species.[8] This is due to the surface layer on these nanoparticles which, although not complete, is predominantly negatively charged and as such repels other nanoparticles allowing them to exist in colloidal suspension. In order to attract the species of interest onto the surface of these nanoparticles two approaches can be taken. One is to actively promote chemical adsorption through the formation of a complexing bond to the surface.[9–11] The other approach is to use the electrostatic attraction between the negatively charged surface and a positively charged analyte.[1,12]

In the case of DNA there is very little positive charge on either the bases or the backbone due to the hard phosphate anions which dominate this molecule resulting in DNA having an overall negative charge. In addition DNA does not contain an inherent chromophore, therefore the covalent attachment of a dye label can be used to provide a suitable resonance contribution to the commonly used visible excitation. This is directly analogous to the approach used in fluorescence detection. One of the major advantages of SERRS is that the metal surface is a very effective quencher of fluorescence and as such any fluorescent dye can be used as a SERRS label as long as it is efficiently adsorbed onto the metal surface. In terms of DNA detection this means that the extensive fluorescence chemistry which has been developed for molecular biology over the last 20–30 years can be used for almost all SERRS experiments. Therefore, some of the original SERRS work conducted on oligonucleotides used commercially available fluorophores as a convenient label.[12] These studies found that the chemical nature of these labels had a large impact on the ability of the oligonucleotides to provide SERRS.

The dyes could be broken down into two distinct classes: positively charged dyes and negatively charged dyes. In the case of positively charged dyes it was sufficient to neutralize the phosphate backbone of the DNA through the use of spermine (a naturally occurring polyamine) which would then allow the electrostatic attraction of the species through the positively charged label.[12] In the case of the negatively charged dyes it was found that when spermine was used with the labelled oligonucleotides it was not sufficient to promote effective surface adsorption and this was attributed to the presence of the negatively charged label. In order to overcome this we introduced several modified deoxyuridine nucleosides adjacent to the negatively charged label.[13] The modified nucleosides consisted of 5-aminopropargyl-2′-deoxyuridine which, after synthesis of the probe and attachment of the label, provided a primary aliphatic amine group which was protonated at physiological pH. This then gave rise to an area of positive charge within the DNA species. When this modified oligonucleotide was mixed with spermine, very efficient and quantitative SERRS could be obtained which was again attributed to the presence of the positively charged bases. To date the only investigation into the variation of the architecture of these probes has been to either have 6 amine modified deoxyuridines or none at all.

This study reports the effect of reducing the overall number of modified bases in the labelled oligonucleotide probe and the effect on the subsequent SERRS signal obtained. Five probes were synthesised with the same DNA sequence and a 5′ FAM label. The variation came in the number of propargylamine modified bases on each probe, adjacent to the dye label, as shown in Table 1. Therefore, the difference between 6, 4, 2, 1 and 0 modified bases was assessed to ascertain how many modified bases were required to allow effective surface adsorption of the DNA on the metal surface and give the optimum SERRS signal. To achieve this we analysed each of the five DNA probes by obtaining the SERRS signal of different concentrations of each probe over a range of concentrations spanning 2 orders of magnitude. This allowed the graph of the concentration dependence of the signal of each probe to be obtained, as shown in Fig. 1 (each point is the average of 5 replicate measurements). The graph shows that the data obtained had an approximately linear concentration dependence and it can

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Table 1 Sequences of bases in the modified oligonucleotides

Number of propargylamines	Sequence 5′ → 3′[a]
0	FAM TCC ACG TTT TCC CAG TCA CGA CGT
1	FAM XC TCC ACG TTT TCC CAG TCA CGA CGT
2	FAM XCXC TCC ACG TTT TCC CAG TCA CGA CGT
4	FAM XCXCXCXC TCC ACG TTT TCC CAG TCA CGA CGT
6	FAM XCXCXCXCXCXC TCC ACG TTT TCC CAG TCA CGA CGT

[a] X denotes propargylamine modification.

be clearly seen that the intensity of the SERRS signal obtained increases with the number of propargylamine modifications present.

The data can equally be presented in a convenient bar chart form where it becomes clear that the largest signals occur at the higher concentration although there is some signal obtained from the unmodified FAM labelled oligonucleotide (Fig. 2). The small signal which is obtained from the unmodified FAM can be attributed to the neutralisation effect of the spermine aggregating agent on the phosphate backbone of the DNA and the negatively charged label, allowing some of the labelled oligonucleotide to come down on the metal surface and give a signal. However, when propargylamine modified bases are added the signal starts to increase, in fact the addition of only one modified base more than doubles the signal obtained. This increase in signal continues with the addition of a second modified base with the largest increases observed when the probes are modified with 6 or 4 modified bases.

As the concentration is reduced the difference between 6 and 4 modifications remains very similar and as such there is no real need to having 6 modified bases since 4 will give the same analytical response. Indeed, at lower concentrations 2 modified bases were sufficient to generate a very similar response to that obtained from the 4 or 6 modified base oligonucleotides.

The detection limits obtained for each of the labelled oligonucleotides are given in Table 2. It can be seen that improved detection limits were achieved immediately upon the addition of only one propargylamine base, with a 3-fold increase in signal intensity. The detection limit obtained for the addition of 1 or 2 and 4 or 6 propargylamine bases are the same owing to the slope of the calibration lines being the same. However, it can be clearly seen that the increase in signal intensity obtained upon addition of 2 modified bases is the most dramatic, with 5 times the signal intensity of the unmodified probe. Therefore, the addition of 2 propargylamine bases is the preferred modification to achieve the best increase in signal intensity and detection limit offset against the increase in cost associated with the addition of additional bases.

This is a significant finding since the addition of modified bases add complexity and, perhaps more importantly, cost to these types of analytical probes. Reducing the amount of propargylamine modifications on a probe sequence simplifies and significantly lowers the cost of the probe. In order to maximise the multiplexing potential of SERRS a large library of available dyes is required and as such negatively charged dyes are necessary as much as positively charged dyes. Although the amount of required modified bases may vary depending on the dye label used, these results indicate that by reducing the number of modified bases to 2, an improvement in SERRS response is possible and allows much simpler probe design for future SERRS based experiments.

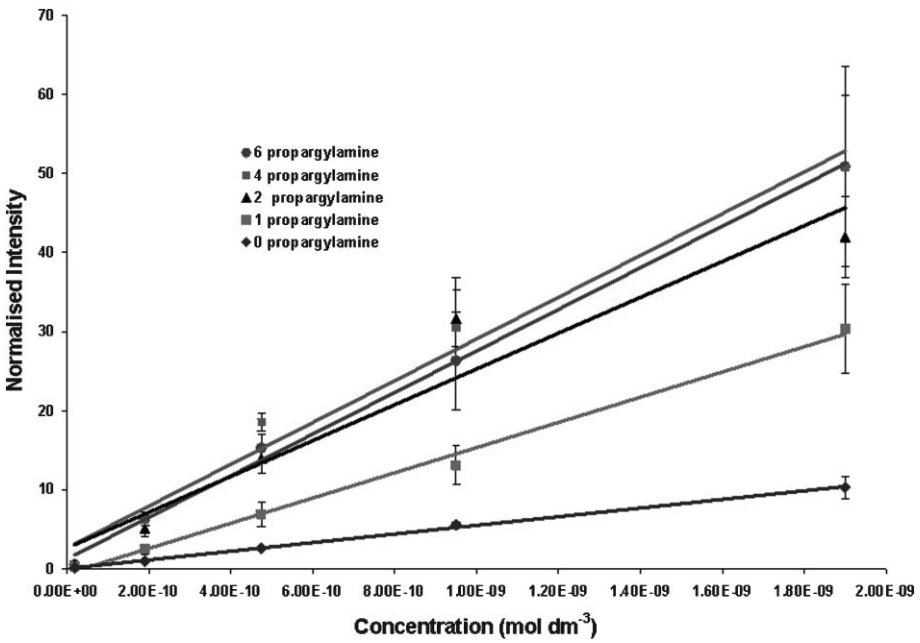

Fig. 1 Concentration dependence of the signal obtained from a FAM labelled oligonucleotide which has been modified with 0, 1, 2, 4 or 6 propargylamine modified bases.

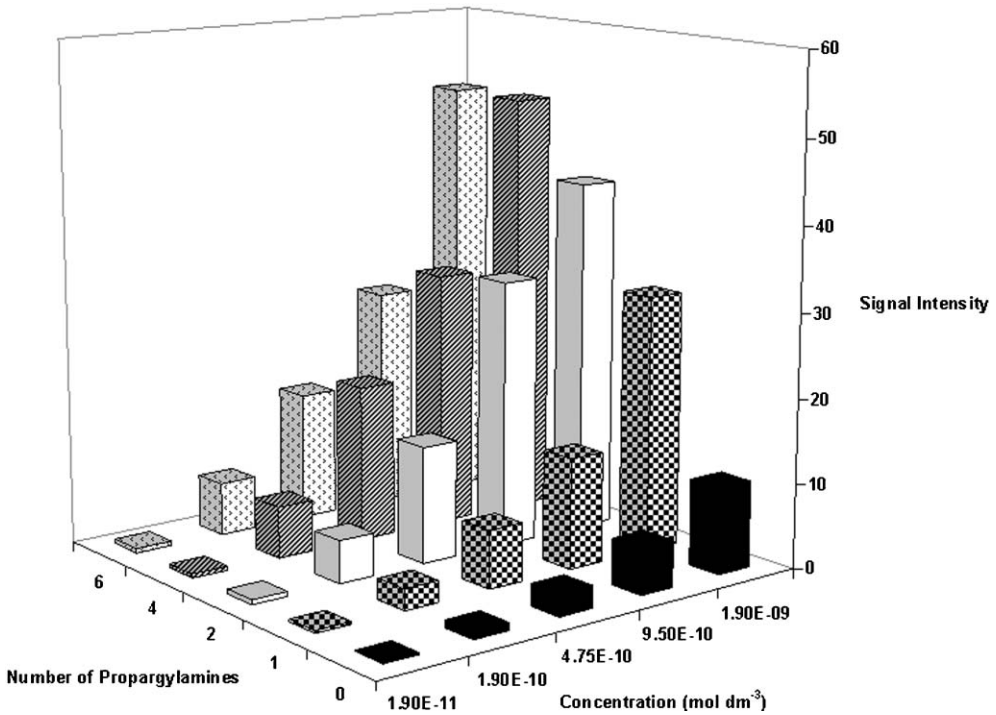

Fig. 2 Concentration dependence of the signal obtained from a FAM labelled oligonucleotide which has been modified with 0, 1, 2, 4 or 6 propargylamine modified bases expressed as a bar chart.

Table 2 Limit of detection of the 5 labelled oligonucleotides

Number of propargylamines	Limit of detection/mol dm^{-3}
0	7.42×10^{-12}
1	1.86×10^{-12}
2	1.86×10^{-12}
4	1.24×10^{-12}
6	1.24×10^{-12}

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