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Conclusions

A series of 5'-dienyl modified oligonucleotides have been synthesised and characterised. The dienyl modifications have been shown in UV-melting experiments to have no effect on the oligonucleotides' ability to hybridise. These oligonucleotides have been successfully reacted with a maleimido modified Tat peptide for biomolecule conjugation *via* Diels-Alder cycloaddition. A 5'-cyclohexadienyl modified oligonucleotide sequence was shown to be most effective in this type of conjugation, affording the desired conjugate in over 70 % yield.

5'-cyclohexadienyl modified oligonucleotides carrying a fluorescent label have been synthesised and characterised. These were used for conjugation to maleimido modified Tat peptide *via* Diels-Alder cycloaddition in order to assess the cellular uptake of the conjugates by fluorescence analysis. However, the yields of these conjugation reactions were very low. It was thought that the cyclohexadiene ring could be involved in π-bonding to the highly conjugated labels, thereby preventing its participation in the Diels-Alder reaction.

A successful route, using palladium chemistry, to mid-sequence dienyl modification of oligonucleotides has been developed. Mid-sequence furanyl and mid-sequence cyclohexadienyl modified oligonucleotides have been synthesised and characterised. Diels-Alder cycloaddition of mid-sequence cyclohexadienyl modified oligonucleotide to maleimido modified Tat peptide afforded the oligonucleotide peptide conjugate in good yield. However, as was observed for 5'-cyclohexadienyl

modified oligonucleotides, conjugation of labelled mid-sequence cyclohexadienyl modified oligonucleotides to Tat peptide was less successful.

As such, it was concluded that the Diels-Alder cycloaddition is not a valid method for conjugation of Tat peptide to labelled oligonucleotides for detection in cell samples.

A DNA sequence with potential biocatalytic properties for the Diels-Alder reaction has been identified, by way of a SELEX-type selection process using a cyclohexadienyl modified DNA aptamer library and biotin maleimide bound to a streptavidin matrix. Tests to evaluate the biocatalytic potential of this sequence are ongoing.

Oligonucleotides have been successfully conjugated to Tat peptide *via* gold nanoparticles. A PEGylated, disulfide functionalised ligand has been synthesised, characterised and used for covalent attachment of Tat peptide to gold nanoparticles. A method for quantification of Tat peptide attached to gold nanoparticles, by enzyme hydrolysis using trypsin, has been developed.

Gold nanoparticles have been successfully bi-functionalised with this ligand and oligonucleotides. A method for quantification of oligonucleotides conjugated to gold nanoparticles, again by enzyme hydrolysis, using the DNAse-1 enzyme, has been developed. Tat peptide has been conjugated, *via* the ligand, to the bi-functionalised nanoparticles for conjugation to oligonucleotides *via* gold nanoparticles. The success of this conjugation was analysed by Tat peptide quantification by trypsin hydrolysis, and confirmed by particle size and zeta potential measurements of the conjugates.

The oligonucleotide Tat peptide nanoparticle conjugates were shown in hybridisation studies to be able to hybridise successfully to a complementary oligonucleotide sequence. This opens up the possibility for their use as bioanalytical probes for diseases, with the ability to cross cell membranes.