2

Research aims

Oligonucleotide conjugation to cell penetrating peptides is an attractive prospect for increasing their cellular uptake; poor cellular uptake of oligonucleotides limits their use as bioanalytical probes for disease. There have been several reports in the literature of methods for oligonucleotide conjugation to peptide sequences, but oligonucleotide conjugation to cell penetrating peptides in particular is more difficult – mostly due to their highly cationic nature. Oligonucleotides are frequently conjugated to the cell penetrating Tat peptide *via* a disulfide linkage. However, this linkage is subject to cleavage by reduction. This research examines novel approaches to oligonucleotide conjugation to Tat peptide.

The Diels-Alder cycloaddition will be examined for this purpose. The Diels-Alder reaction is fast, chemoselective and enhanced in aqueous media and is thereby ideal for biomolecule conjugation. Modification of oligonucleotides with a series of different dienes at both the 5'- and mid-sequence positions will be performed, by synthesis of a series of unique dienyl modified phophoramidites and the effect of these modifications on the oligonucleotides' ability to hybridise with a complementary sequence will be examined to ensure their functionality as bioanalytical probes. The different dienyl modified oligonucleotides will be reacted with a maleimido modified Tat peptide derivative in Diels-Alder cycloadditions for biomolecule conjugation and their reactivities will be compared. Conjugation of labelled dienyl modified oligonucleotides to Tat peptide *via* Diels-Alder cycloaddition will be also be examined, for the purposes of assessing cellular uptake

of the oligonucleotide Tat peptide conjugates by fluorescence analysis of cell assays. Ultimately, bioconjugation of Tat peptide to a molecular beacon *via* the Diels-Alder cycloaddition should be investigated, for generation of a functional probe for disease.

An RNA sequence has been implicated in biocatalysis of the Diels-Alder reaction, accelerating the reactions of anthracene modified oligonucleotides by up to 20,000-fold. [(1)] An investigation into the generation of a DNA sequence with potentially similar biocatalytic properties for the Diels-Alder reaction will be performed; a SELEX-type selection process using a cyclohexadienyl modified aptamer library will be used for this investigation.

The possibility of oligonucleotide conjugation to Tat peptide *via* gold nanoparticles for use as bioanalytical probes will be examined. Synthesis of a suitable ligand for conjugation of the highly cationic Tat peptide to gold nanoparticles will be carried out and a method for quantification of Tat peptide conjugated to nanoparticles will be developed. A route to bi-functionalisation of gold nanoparticles with both oligonucleotides and Tat peptide for oligonucleotide peptide conjugation will be determined; a method for quantification of oligonucleotides in these conjugates will also be developed. Biomolecule quantification of these conjugates as evidence for successful oligonucleotide conjugation to Tat peptide *via* gold nanoparticles will be supported by particle size and zeta potential measurements. The functionality of these conjugates as bioanalytical probes will be examined by way of hybridisation studies.

1. Seelig, B., Jaschke, A., *Chem. Biol.*, **1999**, *6*, 167 – 176.