

Fluorescent Sensors for Triplex DNA

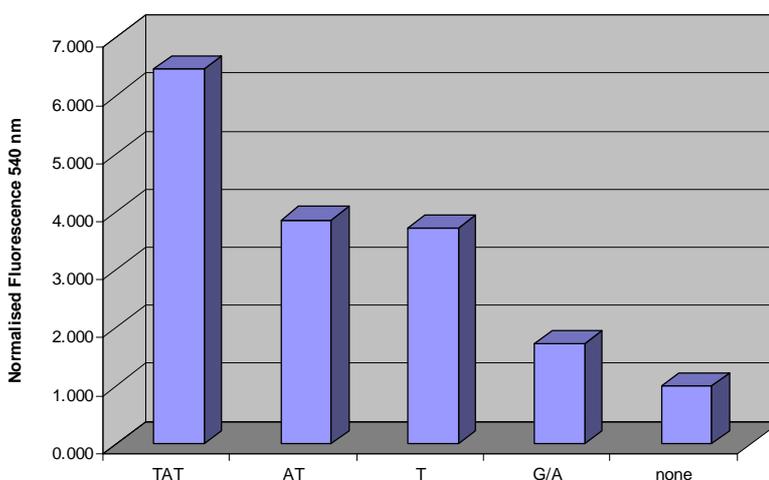
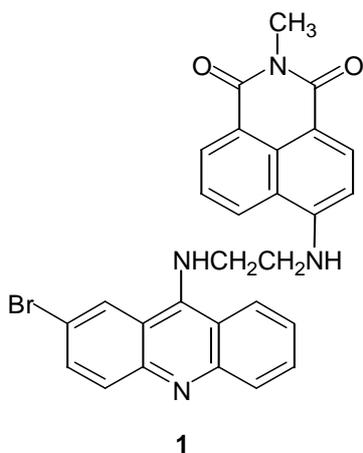
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The aim of the project is to develop a reliable, safe and low cost method for the study of triplex DNA. The project will involve the computer aided design, chemical synthesis and evaluation of a fluorescent reporter system (FRS) with specificity for triplex DNA.

The sequencing of the human genome has provided an invaluable resource to support the study of genes (genomics) and the products of their expression (proteomics). The use of triplex forming oligonucleotides (TFOs) allows potential sequence specific targeting of genes in humans and in other organisms to alter gene expression. Use of TFOs may eventually provide a method to combat diseases that are recalcitrant to current therapies or where no efficacious medicine currently exists.

Various methods exist for the study of DNA triplexes formed by the targeting of synthetic TFOs to duplex DNA sequences (Sørensen et al 2004). Of these, variable temperature UV analysis (VT-UV) and non-denaturing (ND-PAGE) are most common. There are many examples where stable DNA triplexes form but the lack of temperature dependant changes in hyperchromicity renders them "invisible" to VT-UV analysis (Parel & Leumann 2001). Use of ND-PAGE poses an inherent risk to safety and impacts on the environment due to the handling and disposal of radioactive material containing ^{32}P or other radionuclide.

A reliable, safe and low cost method of triplex DNA detection will involve attachment of a fluorophore to an intercalator, specific for triplex DNA. By linking 8-bromo-9-chloroacridine to 4-bromo-*N*-methylnaphthalimide through an ethylenediamine spacer, we have prepared our first FRS compound (**1**) (below, left). Compound (**1**) has marked selectivity for triplex DNA compared with duplex or single stranded DNA, as evidenced by photo-induced electron transfer (PET) enhancement assay (below, right). We now wish to build upon this success through the chemical modification of the acridine portion in compound (**1**) to achieve optimal triplex DNA recognition. Computer aided design (CAD) will allow prediction of better patterns of substitution (Stretowski et al 2005) to promote triplex DNA binding and eliminate binding to duplex DNA. The new FRS compounds, identified by CAD, will then be prepared by chemical synthesis using existing methods, and their interactions with various nucleic acid structures will be determined by PET assay.



- Parel & Leumann (2001) Triple-helix formation in the antiparallel binding motif of oligodeoxynucleotides containing N^9 - and N^7 -2-aminopurine deoxynucleosides. *Nucleic Acids Res.* **29**:2260-2267.
Sørensen et al (2004) Solution structure of a dsDNA:LNA triplex. *Nucleic Acids Res.* **32**:6078-6085.
Stretowski et al (2005) New triple-helix DNA stabilising agents. *Bioorg. Med. Chem. Lett.* **15**:1097-1100.