Size-Expanded DNA Structures

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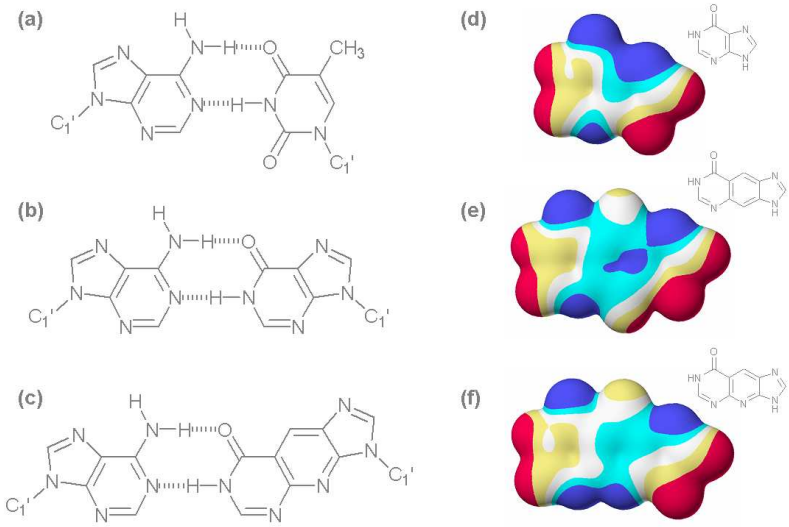
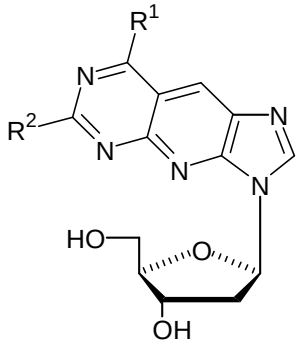
The aim of this project is to develop a new type of triplex forming oligonucleotide (TFO) incorporating size-expanded analogues of the natural DNA bases. The project will involve the chemical synthesis and evaluation of new pyridine-stretched oligonucleotides (PSOs), and computer-assisted modeling of their DNA molecular recognition properties.

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 TFOs have been prepared and studied in attempts to overcome the poor hybrid stability of triplex structures formed between TFO and duplex DNA target (Sørensen et al 2004). Recognition of target duplex DNA relies on Hoogsteen hydrogen bond formation and is largely restricted to contiguous polypurine DNA target sequences; a serious handicap compared with the targeting of mRNA by antisense oligonucleotides (Buchini & Leumann 2003).

We aim to further develop (Crawford et al 2009) a useful synthetic route to prepare compounds (**1**), (**2**) and (**3**) (below, right) that are size-expanded analogues of adenine (A), hypoxanthine (H) and diaminopurine (D). We have demonstrated that oligonucleotides incorporating (**1**), for example, maintain Watson-Crick base pairing potential similar to the DNA bases and can form duplex structures that are significantly more stable than natural DNA (below, left). We now wish to build upon this success by exploiting the enhanced DNA molecular recognition properties of such PSO structures to target duplex DNA more effectively. Computer assisted modeling (CAM) will allow us to predict better PSO structures that might involve analogues such as (**4**) and (**5**) with enhanced triplex stability. The new PSOs, identified by CAM, will be prepared by chemical synthesis using our existing routes, and their interactions with various nucleic acid structures determined by in-house assay methods.





(**1**) $R^1 = NH_2$, $R^2 = H$ (strA)
(**2**) $R^1 = OH$, $R^2 = H$ (strH)
(**3**) $R^1 = NH_2$, $R^2 = NH_2$ (strD)
(**4**) $R^1 = OH$, $R^2 = NH_2$ (strG)
(**5**) $R^1 = NH_2$, $R^2 = OH$ (strI)

Buchini & Leumann (2003) Recent improvements in antigene technology. *Curr. Opin. Chem. Biol.* **7**:717-726.
Crawford et al (2009) Preparation of 9-substituted pyridine-stretched adenines and hypoxanthines. *Synthesis* 1271-1278.
Sørensen et al (2004) Solution structure of a dsDNA:LNA triplex. *Nucleic Acids Res.* **32**:6078-6085.