

## Locked Nucleic Acids (LNAs)

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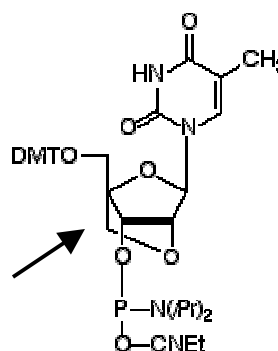
### Introduction

With few exceptions, experimental success in molecular biology is dependent upon specific, discriminating, and persistent hybridization events involving synthetic oligonucleotides and their complementary target sequences. While unmodified oligodeoxynucleotides will routinely form desired DNA:DNA and DNA:RNA duplexes, synthesis of various modifications that confer enhanced high-affinity recognition of DNA and RNA targets has been an ongoing endeavor. A variety of nucleic acid analogs have been developed that display increased thermal stabilities when hybridized to with complementary DNAs or RNAs as compared to unmodified DNA:DNA and DNA:RNA duplexes. Among these analogs are peptide nucleic acids (PNAs)(Hyrup and Neilson, 1996; Nielson and Haaima, 1997), 2'-fluoro N3-P5'-phosphoramidites (Schulz and Gryaznov, 1996), and 1', 5'- anhydrohexitol nucleic acids (HNAs)(VanAerschot et al., 1996; Hendrix et al., 1997). While such analogs succeed to varying degrees in achieving increased thermal stabilities, they fail to provide enhanced target recognition. A relatively benign modification that has demonstrated both of these desirable qualities is the **Locked Nucleic Acid (LNA)**.

### LNA Thermodynamics

Singh et al. (1998) and Obika et al. (1998) reported on a minimal alteration of the pentose sugar of ribo- and deoxyribonucleotides that constrained, or "locked," the sugar in the N-type conformation seen in A-form DNA. The lock was achieved via a 2'-O, 4'-C methylene linkage in 1,2:5,6-di-O-isopropylene- $\alpha$ -D-allofuranose. This alteration then served as the foundation for synthesizing locked nucleotide phosphoramidite monomers (Fig. 1). Oligonucleotides containing one or more of these monomers were given the name lock nucleic acid, LNA (Koshkin et al, 1998).

Figure 1. Structure of a 2'-O, 4'-C methylene bicyclonucleoside monomer or "locked nucleic acid" (LNA) phosphoramidite. The single structural variation is indicated.



LNAs were immediately seen to display remarkably increased thermodynamic stability and enhanced nucleic acid recognition. Initial investigations of LNA melting temperatures ( $T_m$ ) by Koshkin et al. (1998) revealed increased values per LNA monomer ( $\Delta T_m$ ) of +3 to +5°C and +4 to +8°C against complementary DNA and RNA oligonucleotides respectively. Subsequent examination of various LNA constructs indicated that the presence of a locked nucleotide influences the conformational status of adjacent unmodified nucleotides such that the N-type conformer is favored over the S-type conformer in those bases as well (Petersen et al., 2000). Also, Kvaerno and Wengel (1999) synthesized an abasic LNA monomer, a 1-deoxy-2-O, 4-C-methylene-D-ribofuranose, that was used by them to examine the thermodynamic properties of the modified sugar alone against complementary DNA oligonucleotides. Their results indicate that the conformational restriction of the pentofuranose and backbone alone will not induce an increase in  $T_m$ . Thus, they conclude, the nucleobase is essential as a mediator of the conformational change (Kvaerno and Wengel, 1999: 658).

These early studies suggested that LNAs could potentially be important tools for a wide range of molecular applications (cf., Braasch and Corey, 2001; Petersen and Wengel, 2003). Numerous recent reports have confirmed both the utility and the versatility of LNAs (see below) and a refined thermodynamic study of LNA-DNA duplex formation by McTigue et al. (2004) has contributed even greater precision to LNA design parameters.

McTigue et al. (2004) examined hybridization entropy ( $\Delta H^\circ$ ) and enthalpy ( $\Delta S^\circ$ ) as well as  $T_m$  for 100 LNA-DNA duplexes containing a single internal LNA nucleotide. Their results show that LNA pyrimidines contribute more stability than do LNA purines with average  $\Delta T_m$  values of  $4.44 \pm 1.46^\circ\text{C}$  for LNA-C,  $3.21 \pm 1.41^\circ\text{C}$  for LNA-T,  $2.83 \pm 1.75^\circ\text{C}$  for LNA-G, and  $2.11 \pm 1.30^\circ\text{C}$  for LNA-A. Further, the observed range of  $\Delta T_m$  for each LNA nucleotide is context dependent with both 5' and 3' unmodified neighbors influencing stability. Interestingly, purine neighbors appear to make the more substantial difference in stability. Their observations of  $\Delta\Delta H^\circ$  and  $\Delta\Delta S^\circ$  in their 100 constructs provide a more accurate overall  $T_m$  estimate for LNAs than previously available.

## LNA Applications

Enhanced nucleic acid recognition by LNA-containing oligonucleotides makes them desirable for a host of molecular applications. Kurreck et al. (2002) reported on the design of various chimeric LNA-DNA antisense constructs. Substantial  $\Delta T_m$  values were achieved with both interspersed designs and LNA-DNA-LNA gapmers. Grunweller et al. (2003) compared LNA-DNA-LNA gapmers with siRNAs, phosphorothioate and 2'-O-methyl RNA-DNA gapmers against expression of the vanilloid receptor subtype 1 (VR1) in Cos-7 cells. Their results show that siRNAs are the most potent antisense molecules against VR1 expression but they also found that LNA-DNA-LNA gapmers having a 5nt-8nt-5nt design were 175- and 550-fold superior in suppressing VR1 compared to isosequential phosphorothioate and 2'-Ome oligonucleotides respectively.

Ugozzoli et al. (2004) have successfully employed LNA-containing dual-labeled probes in Real-Time PCR assays of human  $\beta$ -globin mutations and of mutations in the thrombotic risk genes factor V Leiden (FVL) and prothrombin. Compared with isosequential DNA probes, LNA probes demonstrated superior allelic discrimination. Thus, since  $T_m$ s equivalent to DNA probes can be achieved with shorter LNA probes and shorter probe sequences are more sensitive to single-base mismatches, these constructs are generally useful for genotyping assays. Indeed, a number of SNP applications incorporating LNAs have validated this assertion (cf., Johnson et al., 2004; Latorra et al., 2003a; Simeonov and Nikiforov, 2002).

Numerous reports on the use of LNA microarrays have been presented at various conferences and several research applications are soon to appear (cf., Tolstrup et al., 2003).

Finally, DNA triplex formation is another avenue that has been pursued in gene silencing but with only moderate success. The primary reason for this has been the nature of DNA triplex formation itself. The target dsDNA sequence is Watson-Crick bonded and the triplex forming oligodeoxynucleotides (TFOs) bind to the duplex via Hoogsteen hydrogen bonding; viz., T-A:T and C<sup>+</sup>-G:C triplets. This strategy is limited by the fact that only purine-pyrimidine dsDNA can be targeted and that the cytosines in the TFO must be protonated. This last limitation is due to the requirement for acidic conditions in the assay. Petersen et al. (2003) reported that LNA-containing TFOs will stabilize triplex formation at physiologic pH. A 15-mer containing seven LNAs raised the temperature for triplex to duplex transformation from 33°C to ~60°C at pH 6.8 (Torigoe et al., 2001). Petersen et al. note, however, that an all-LNA TFO will not form triplexes under any conditions.

### **LNA Synthesis at IDT**

IDT offers synthesis of LNAs under license from Exiqon A/S (Denmark). LNA phosphoramidites can be placed either 5' or internally. 3' placement of LNAs is not currently offered. Note that the substantial increases in  $T_m$  conferred by LNAs increases the likelihood of stable secondary structure formation in the form of hairpins and dimers. LNA design issues have been specifically addressed by Latorra et al., (2003b) and Braasch et al. (2002).

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