



Locked and Unlocked Nucleosides in Functional Nucleic Acids

Doessing, Holger; Vester, Birte

Published in:
Molecules

Link to article, DOI:
[10.3390/molecules16064511](https://doi.org/10.3390/molecules16064511)

Publication date:
2011

Document Version
Publisher's PDF, also known as Version of record

[Link back to DTU Orbit](#)

Citation (APA):
Doessing, H., & Vester, B. (2011). Locked and Unlocked Nucleosides in Functional Nucleic Acids. *Molecules*, 16(6), 4511-4526. <https://doi.org/10.3390/molecules16064511>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Review

Locked and Unlocked Nucleosides in Functional Nucleic Acids

Holger Doessing and Birte Vester *

Nucleic Acid Center, Department of Biochemistry and Molecular Biology, University of Southern Denmark, Campusvej 55, Odense M 5230, Denmark; E-Mail: holgerdoessing@gmail.com (H.D.)

* Author to whom correspondence should be addressed; E-Mail: b.vester@bmb.sdu.dk;
Tel.: +45-6550-2406; Fax: +45-6550-2467.

Received: 3 May 2011; in revised form: 19 May 2011 / Accepted: 25 May 2011 /

Published: 27 May 2011

Abstract: Nucleic acids are able to adopt a plethora of structures, many of which are of interest in therapeutics, bio- or nanotechnology. However, structural and biochemical stability is a major concern which has been addressed by incorporating a range of modifications and nucleoside derivatives. This review summarizes the use of locked nucleic acid (LNA) and un-locked nucleic acid (UNA) monomers in functional nucleic acids such as aptamers, ribozymes, and DNAzymes.

Keywords: locked nucleic acid (LNA); unlocked nucleic acid (UNA); aptamer; ribozyme; deoxyribozymes (DNAzyme)

1. Introduction

Ribozymes, DNAzymes, and aptamers, collectively referred to as ‘functional nucleic acids’, are RNA or DNA structures with sequence-specific folds. These functional nucleic acids achieve their tertiary folds and activity through a combination of different molecular interactions and motifs: Hydrogen bonds, hydrophobic interactions, van der Waals forces, canonical and non-canonical base pairs, base stacking, coaxial stacking, tetraloops, G-quadruplexes, and metal ion coordination [1,2]. However, the use of nucleic acids in therapeutics and bio- and nanotechnologies is troubled by denaturation and/or biodegradation of the nucleic compounds. Non-natural nucleosides may offer improved half-life *in vivo*, better structural stability, or novel interacting groups. 2'-modified ribonucleoside analogues have attracted interest, as modification to this position on the ribose often confers improved nuclease resistance and allows fine-tuning of the helical structure. ‘Locked’

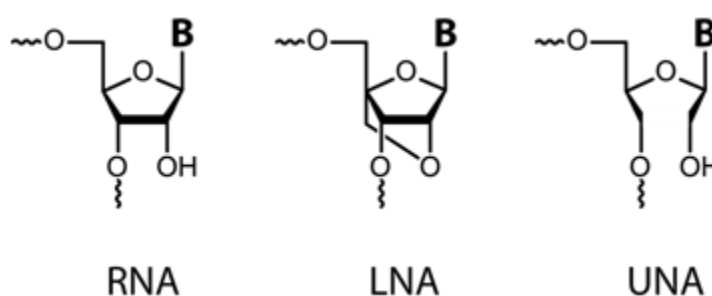
ribonucleoside analogues [3-5] in particular have gained interest over the last decade, and the recent advent of the conceptually opposite ‘unlocked’ nucleoside analogues [6,7] have expanded the biochemists’ tool set even further.

2. Locked Nucleic Acids

Structure and Properties

A locked nucleic acid (LNA) is a ribonucleoside homologue that features a 2'-O,4'-C-methylene linker or bridge (Figure 1) [3-5,8]. This locks the ribose moiety in the C3'-endo conformation and makes LNA an RNA mimic. In duplexes, LNA causes a local re-organization of the phosphate backbone, including pucker steering of its neighbouring 3' nucleotides towards the A-form, and replacing every third residue or more with its corresponding LNA moiety yields a near-canonical A-form heteroduplex [9]. By pre-arranging the nucleobases for better stacking, the enthalpy loss is increased upon duplex formation, while simultaneously reducing entropy loss [10]. This translates into very high thermal stability of LNA-modified duplexes. Introduction of LNA into the DNA strand of a DNA:RNA duplex has been shown to increase the T_m between 1–8 °C per each LNA moiety incorporated (compared to the unmodified duplex) [3,11-13]. Modification of the RNA strand in a similar heteroduplex has led to increases in T_m of no less than 2–10 °C per LNA moiety [3,11-15]. This property of LNA has been put to use in applications where high affinity is desirable, such as gene silencing [16], modulation of RNA splicing [17-20], RNA interference [21], molecular beacon probes [22-25], miRNA detection [reviewed in 26] and DNAzymes [27-37].

Figure 1. Structures of RNA, LNA, and UNA monomers. LNA is ‘locked’ in the RNA-like C3'-endo conformation due to the methylene linker. UNA lacks the C2'-C3' bond and is therefore acyclic and ‘unlocked’.



The toxicity of LNA has been assessed in several studies: No changes were observed in body temperature and histological preparations of brains from rats injected with antisense LNA oligonucleotides [38], and mice repeatedly dosed with an LNA antisense oligomer at 1–2 mg/kg [39], 5 mg/kg [40], or 25 mg/kg [17,41] showed no serum liver cytotoxicity markers at this level. Stein *et al.* [42] maintained LNA antisense-mediated gene silencing in cell cultures for >240 days without observing any toxic side-effects. Swayze *et al.* [43] found, however, that mice treated repeatedly with LNA oligonucleotides experienced significant weight loss and were adversely affected, as judged from enzymatic assays and histopathological analyses of liver sections. Given the strong base-pairing potential of LNA-modified antisense oligonucleotides off-target effects might

explain these results. Santaris Pharma A/S currently has LNA-based drugs against e.g., hepatitis C or tumor growth, in clinical trials [44,45].

One of the motivations for employing LNA is its stability towards nucleases. Short interfering RNAs (siRNA) are rapidly degraded by blood ribonucleases, but introduction of two LNA moieties at both 3' termini has been shown to offer protection in human serum beyond 6 hours, a 4-fold improvement over the unmodified siRNA. Replacing one strand with a DNA/LNA mix-mer further improved stability beyond 48 h. Similar results were obtained in 10% foetal bovine serum and 100% mouse serum [46], as well as in live mouse blood [47] and with similar oligo designs [48].

Deoxyribonuclease activity in blood is generally believed to be shared among deoxyribonuclease I (DNase I), DNase II, and phosphodiesterase I with the former being the main component. Two or more LNA moieties towards the 3' end can offer efficient protection against snake venom phosphodiesterase I (SVPD) [49]. SVPD is, however, able to completely digest substrates of a scattered arrangement with singular LNAs [50,51]. DNase I is an endonuclease that cleaves single- or double-stranded DNA adjacent to pyrimidines. Remarkably, by using 5' and 3' terminal LNA nucleosides together on both strands in a DNA duplex Crinelli *et al.* [52] saw a marked improvement in stability over that observed with the unmodified DNA duplex. Internal modifications did not improve the nuclease resistance further, though. In human serum the increase on DNA oligomer half-life varies with the number of terminal LNA moieties, from 2.5-fold (1 LNA at either end), 11-fold (3 LNA monomers at both ends) to 15-fold (4 LNA monomers at either end) [14]. Further gains can be achieved with mix-mer designs, which have been shown to yield 30-fold improvements in half-life [38]. Together, these reports leave little doubt that modification with LNA offers tremendous advantages regarding stability of therapeutic oligomers.

3. Functional Nucleic Acids with LNA

3.1. LNA in Aptamers

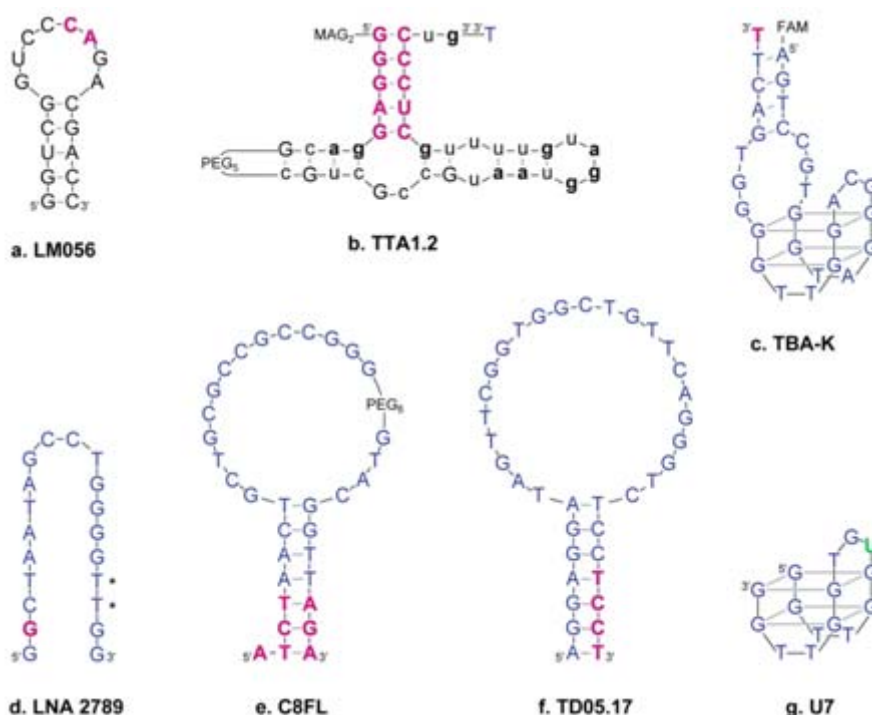
Aptamers are nucleic acid oligonucleotides, whose sequence allows them to fold into defined tertiary structures that act as ligands and bind their corresponding target molecule with specificity and affinity rivalling that of antibodies. Aptamers have been raised against metal ions, small organic dyes, neurotransmitters, nucleotides, cofactors, amino acids, oligonucleotides, carbohydrates, antibiotics, many proteins, anthrax spores, and live cells. A more comprehensive list is provided elsewhere [53]. The potential uses of aptamers are numerous and include applications in purification, sensors, diagnostics, and therapeutics. Further information can be found in excellent reviews, e.g., [54-56] and references highlighted therein. However, to date, only 14 aptamer-based drugs have entered clinical trials [55].

One of the first reports of LNA modification to an RNA aptamer was that of the HIV-1 *trans*-activation responsive (TAR) RNA 'aptamer' R06 that disrupts the TAT-Tar interaction by forming a 'kissing loop' complex with the TAT RNA [57]. As its binding mode consists of base-pairing of six nucleotides in the apical loop to the TAR RNA it can be argued that this is not a true aptamer but a cross-over to the field of antisense oligomers. LNA modifications were placed within these six nucleotides, and only mix-mer designs afforded binding [58]. A complete screening of all possible

LNA/2'-O-methyl modifications to the loop later yielded optimized structures with ~20-fold improvements in K_d into the sub-nanomolar range (Figure 2a) [59].

The first example of an LNA-modified aptamer not binding through base pairing with the target was with the 2'-F pyrimidine RNA aptamer 'TTA1'. This 39-mer aptamer forms a three-way junction and binds human glycoprotein Tenascin-C, which resides in the extracellular matrix [60]. LNA moieties were permitted in only one of the aptamer's supposed three stems, and together with 5'-conjugation to a 2-mercaptoacetylglycyl-glycyl (MAG₂) chelator for attachment of technetium-99m this lead to increased serum stability and improved tumour uptake and blood retention in tumour-bearing nude mice (Figure 2b) [61].

Figure 2. Presumed secondary structures of various modified aptamers. RNA, black; DNA, blue; LNA, purple; UNA, green. (a). Aptamer against HIV-1 trans-activation responsive (TAR) element [59]; (b). Human Tenascin-C-binding aptamer [61]. Lowercase boldface, 2'-OCH₃-modification; lowercase non-bold, 2'-F-modification; (c). Thrombin-binding aptamer [62], the structure of this aptamer remains elusive. FAM, fluorescein label; (d). Avidin-binding aptamer [63]. Asterisks indicate sites that allow for modification with 2'-amino-LNA; (e). Aptamer against T-cell leukaemia (CCRF-CEM) cells [64]; (f). Aptamer against the mIgM BCR epitope on B-cells [65]; (g). Thrombin-binding aptamer modified with UNA [66].



The first DNA aptamer ever to be identified, the thrombin binding aptamer (TBA) [67], was modified with a single LNA G substitution at the 3' terminus. This led to decreased thrombin binding [68], and subsequent attempts to introduce LNA to the aptamer's G-quadruplex structure yielded no improvements over the unmodified aptamer thereby demonstrating how this aptamer depends on an induced fit that is incompatible with LNA [69]. Kasahara *et al.* [62] found that 3'-end capping of a very similar thrombin-binding aptamer [70] with LNA had little effect on binding affinity, though (Figure 2c).

A similar study on an avidin-binding DNA aptamer was recently published by our lab. The previously un-optimized aptamer [71] was truncated from 61 to 21 nucleotides, increasing the K_d 10-fold. The aptamer was then screened for various single and dual LNA substitutions, and one substitution restored the K_d to that of the original aptamer. 2'-*O*-methyl substitution at this site did not yield the same effect. Further introduction of T-2'-amino-LNA caused only a small increase in K_d , suggesting that this aptamer was very tolerant towards LNA modifications in general (Figure 2d) [63].

Shangguan *et al.* [64] truncated aptamer 'sgc8' against T-cell leukaemia cell line CCRF-CEM while retaining its K_d . Various LNA substitutions were tested and LNA was accepted in the terminal stem region. This modification, along with replacing a presumed single-stranded region with a polyethylene glycol linker, yielded an aptamer with markedly improved serum stability and a K_d ~2-fold higher than that of the original aptamer (Figure 2e).

The most recent example of an LNA-modified aptamer is the modification of DNA aptamer 'TD05', which binds a B-cell surface epitope dubbed 'mIgM BCR' that is expressed on B-cells and many B-cell lymphomas [72,73]. The aptamer's stem-loop structure was truncated, causing a near 10-fold increase in target affinity, and linking multiple aptamers together yielded up to 5-fold further improvements. Three arrangements of LNA were evaluated and LNA modification of pyrimidines at the base of the stem gave the best results and offered, as expected, prolonged stability in human serum (Figure 2f) [65].

3.2. LNA in Catalytic Nucleic Acids

The term 'ribozyme' refers to well-defined RNA structures with the ability to catalyse specific chemical reactions. The first ribozyme to be discovered was a self-splicing RNA [74,75]. Since then, ribozymes have been identified in a number of various biological settings, as well as in *in vitro* experiments [76]. Deoxyribozymes or 'DNAzymes' are equivalent catalytic DNA structures obtained by *in vitro* selection, of which the first was an RNA-cleaving Pb^{2+} -dependent DNA oligomer [77]. Although the tertiary interactions required for catalysis remain elusive modification with LNA has been investigated.

Catalytic nucleic acids that cleave RNA bind their substrates by Watson-Crick base-pairing and thus seem very well suited to modification with LNA. For instance, the '10-23' DNAzyme [78] was modified to contain two LNA monomers in each of the binding arms, which resulted in much improved cleavage of even a highly structured RNA of 2,904 nucleotides as well as the 17 nucleotide minimal RNA substrate [27,34]. Incorporation of 3-4 LNA moieties into another '10-23' variant yielded similar results against viral RNA and even cleaved otherwise inaccessible targets in highly structured RNA but at the cost of decreased turnover rates [33,36]. A number of studies demonstrating similar modifications to the substrate binding arms have also been published [28-32,34,35,37].

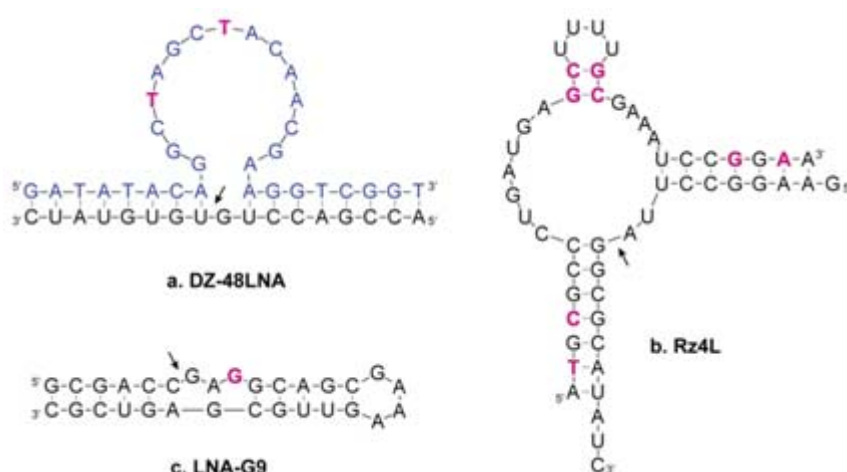
Beneficial modification of the catalytic cores has proven more difficult. Robaldo *et al.* [79] introduced LNA-T into two positions in the '10-23' core and this drastically reduced the activity of the enzyme (Figure 3a). The purported catalytic stem-loop of the '8-17' DNAzyme [78] has been the target of LNA modification in our lab, but this led to inactivation of the modified DNAzyme [80].

Christiansen *et al.* [81] explored LNA incorporation into the 'hammerhead' ribozyme [82] and found that interspersing LNA with DNA in the substrate binding arms increased single turnover rates

20-fold and also improved multiple turnover behaviour. Modifications to ‘stem II’ did not improve the ribozyme, except for one variant that showed a slight improvement in multiple turnover kinetics (Figure 3b). Fedoruk-Wyszomirska *et al.* [83] introduced LNA in one substrate binding arm as well as an auxiliary domain in their hammerhead variant and found that the LNA had no impact on single-turnover rates nor mRNA silencing efficacy in HeLa cells.

Finally, LNA was used to probe the requirements for C3'-endo sugar pucker at three positions in a small, lead-dependent ribozyme [84,85]. This revealed two discrepancies between the inactive ground states recorded by X-ray crystallography and NMR and the catalytically active structure: LNA substitution of a nucleoside believed to be in the C3'-endo pucker caused a 15-fold decrease in self-cleavage activity, whereas LNA substitution at a position believed to exhibit C2'-endo pucker increased the activity 20-fold (Figure 3c) [86].

Figure 3. Secondary structures of various modified catalytic nucleic acids. Arrows indicate backbone cleavage sites. RNA, black; DNA, blue; LNA, purple. (a). ‘10–23’ DNAzyme, these modifications led to reduced activity [79]; (b). Hammerhead ribozyme, the shown modifications led to slightly improved multiple turnover kinetics [81]; (c). Leadzyme, this modification increased the activity 20-fold [86].



4. Unlocked Nucleic Acids

4.1. Properties

Unlocked nucleic acid (UNA) [6,7,87] lacks the C2'-C4' bond normally found in ribonucleosides (Figure 1), and it is therefore highly flexible. The term ‘unlocked’ hence reflects the structural differences from ‘locked’ nucleic acid [88]. This is also mirrored in the destabilizing effects of UNA-incorporation in duplexes, with up to 12 °C decrease in T_m per UNA monomer in RNA:RNA duplexes and 10 °C per UNA monomer in RNA:DNA duplexes [6]. Incubation of a UNA A tri-mer with SVPD or in cell lysate indicates that UNA is also highly resistant to nucleases [89]. UNA monomers have proven very useful in fine-tuning the specificity and potency of siRNA, in particularly in combination with LNA [90-92]. It is noteworthy that Bramsen *et al.* [90] found that even highly

potent UNA-modified siRNAs did not affect cell viability. Rigorous studies on toxicity have not yet been carried out, however.

4.2. UNA in Aptamers

So far, there is only a single published report on UNA-modification to an aptamer: the thrombin-binding DNA aptamer, TBA [67] was systematically substituted with UNA-G or -U monomers. Substitution with UNA-U was tolerated at several positions, and modification to position 7 (Figure 2g) lead to a slightly improved K_d (from 103 nM to 78 nM) and a ~3-fold improvement in antithrombin effect, as judged by a fibrin-clot formation assay [66]. The DNA aptamer adopts a G-quadruplex structure, and this places the UNA-U monomer in the central loop. However, structural data on the precise conformation and orientation of the protein-bound aptamer remain ambiguous [93-95] and preclude any precise rationalizations on the effects conferred by the UNA on this particular aptamer.

5. De Novo Selection of Modified Nucleic Acid Structures

Synthetic functional nucleic acids are identified through an iterative technique known as *in vitro* selection [96-99]. The classic approach involves enriching a sequence pool for members with the desired activity by repeated incubation, amplification, and pool re-generation. Finally, the pool is sequenced. By using a pool of oligomers containing modified nucleosides it is possible to realize functional nucleic acid structures that natively contain these modifications. The use of such nucleosides in classical *in vitro* selection requires that they are tolerated by the enzymes used for amplification and regeneration of the pool [100]. To date, LNA and UNA nucleotides have only been used post-selection. This is probably due to the lack of the appropriate methods for maintaining LNA or UNA-modified pools over successive selection rounds. Phosphoramidite derivatives of all standard nucleobases have been introduced [6,7,101-103], thus making the initial synthesis of such libraries a relatively straightforward process; the lower coupling efficiencies of LNA and UNA should be taken into account, however, and may disfavour synthesis of sequences containing consecutive modifications. Reading of LNA-modified templates, as well as incorporation of triphosphates LNA TTP, LNA ATP, LNA GTP, and LNA 5-methyl-CTP, has been demonstrated with Taq [104], Vent(exo-) [104], Phusion High-Fidelity [104-106], 9° N_m [107,108], and KOD [104,109,110] DNA polymerases, as well as T7 RNA polymerase [107]. The most promising results published so far have been the successful PCR amplification of a 50-nucleotide template while incorporating LNA A at 9 sites [109] and 21 successive incorporations of all LNA nucleosides [110].

Applying LNA to the classical *in vitro* selection setup with many rounds of selection and regeneration remains non-trivial (our unpublished results), and other methods might therefore be considered for selection of LNA-containing structures. Selection by capillary electrophoresis (CE) or ‘non-equilibrium capillary electrophoresis of equilibrium mixtures’ (NECEEM) separates non-binding and binding members in solution and can yield aptamers in as few as 1-4 rounds [111-117] and may even alleviate the need for pool amplification [112,118]. As only a few rounds of selection are required with CE, the pools used rarely converge [113-116]. This is seen as an advantage though, as it indicates that larger sequence spaces are explored with this technique.

In another method the library is synthesized directly onto small beads by a split-and-mix technique so each bead becomes coated in a unique sequence. The library is then screened for beads that retain the (labelled) target, and this approach has been used for finding phosphorothioate [119] or iso-guanine-modified aptamers [67]. The technique is, however, limited by the very small size of the libraries, typically containing only a few thousand unique members.

The enzymatic steps required for pool re-generation and sequencing can be avoided altogether by employing array-based selection. Microarrays have been used for assessing various mutations in a known aptamer [120], and this method also seems well suited for screening for activity of functional nucleic acids modified post-selection. More interestingly, the technique dubbed ‘closed loop aptameric directed evolution’ (CLADE) [121] that uses array-based libraries in combination with *in silico* evolution has been used to generate aptamers from starting pools of 5,500 [121], 46,000 [122], or 150,000 [123] unique members. The initial array seeks to cover a large sequence space, and experimental binding data are then combined to mutate selected members for the next generation array. This approach has yielded aptamers in 4–9 selection rounds and seems suitable for even highly modified libraries. It therefore seems likely that the future application of LNA and UNA in functional acids may be based on either of these inventive approaches.

6. Conclusions

In conclusion, the application of LNA and UNA in functional nucleic acids is still very promising and definitely worth pursuing. The same goes for other LNA derivatives such as amino-LNA [124], which has not been covered by this review. The development of efficient protocols for enzymatic incorporation of modified nucleotides and reading/copying of modified oligonucleotides is reckoned important, specifically for selection of aptamers spiked with LNA, which are predicted to play an important role in diagnostics and biotechnology in the future. As summarized in this review, there are now numerous studies supporting the usefulness of the specific features of LNA and UNA in the field of RNA therapeutics, including aptamers, siRNAs, microRNAs and antagomirs, most of them working by hybridization as a sort of antisense oligonucleotides. *In vitro* selection of function nucleic acids with nucleoside modifications has been very successful with 2'-F pyrimidines and subsequent substitution with 2'-O-methylated purines. Nonetheless, LNA remains a solid candidate for post-selection modification of stem regions and termini, where it offers nuclease resistance and can improve conformational stability. The presence of even a few modifications can also have a large impact on specificity, off-target effects, structure, stability and clearance. Even though the optimal employment of the novel UNA still remains to be seen, it is now largely up to companies to take advantage of both of these nucleoside analogues and develop them for commercial usage.

Acknowledgments

This study was supported by the Danish National Research Foundation. H.D. was funded by a stipend from The Danish Council for Independent Research, Technology and Production Sciences (FTP).

References

1. Hermann, T.; Patel, D.J. Stitching together RNA tertiary architectures. *J. Mol. Biol.* **1999**, *294*, 829-849.
2. Nagaswamy, U.; Voss, N.; Zhang, Z.; Fox, G.E. Database of non-canonical base pairs found in known RNA structures. *Nucleic Acids Res.* **2000**, *28*, 375-376.
3. Koshkin, A.A.; Singh, S.K.; Nielsen, P.; Rajwanshi, V.K.; Kumar, R.; Meldgaard, M.; Olsen, C.E.; Wengel, J. LNA (Locked Nucleic Acids): Synthesis of the adenine, cytosine, guanine, 5-methylcytosine, thymine and uracil bicyclonucleoside monomers, oligomerisation, and unprecedented nucleic acid recognition. *Tetrahedron* **1998**, *54*, 3607-3630.
4. Singh, S.K.; Nielsen, P.; Koshkin, A.; Wengel, J. LNA (locked nucleic acids): Synthesis and high-affinity nucleic acid recognition. *Chem. Commun.* **1998**, *4*, 455-456.
5. Obika, S.; Nanbu, D.; Hari, Y.; Morio, K.-I.; In, Y.; Ishida, T.; Imanishi, T. Synthesis of 2'-O,4'-C-methyleneuridine and -cytidine. Novel bicyclic nucleosides having a fixed C3'-endo sugar puckering. *Tetrahedron Lett.* **1997**, *38*, 8735-8738.
6. Langkjaer, N.; Pasternak, A.; Wengel, J. UNA (unlocked nucleic acid): A flexible RNA mimic that allows engineering of nucleic acid duplex stability. *Bioorg. Med. Chem.* **2009**, *17*, 5420-5425.
7. Nielsen, P.; Dreioe, L.H.; Wengel, J. Synthesis and evaluation of oligodeoxynucleotides containing acyclic nucleosides: Introduction of three novel analogues and a summary. *Bioorg. Med. Chem.* **1995**, *3*, 19-28.
8. Vester, B.; Wengel, J. LNA (locked nucleic acid): High-affinity targeting of complementary RNA and DNA. *Biochemistry* **2004**, *43*, 13233-13241.
9. Nielsen, K.E.; Rasmussen, J.; Kumar, R.; Wengel, J.; Jacobsen, J.P.; Petersen, M. NMR studies of fully modified locked nucleic acid (LNA) hybrids: Solution structure of an LNA:RNA hybrid and characterization of an LNA:DNA hybrid. *Bioconjug. Chem.* **2004**, *15*, 449-457.
10. Petersen, M.; Nielsen, C.B.; Nielsen, K.E.; Jensen, G.A.; Bondensgaard, K.; Singh, S.K.; Rajwanshi, V.K.; Koshkin, A.A.; Dahl, B.M.; Wengel, J.; *et al.* The conformations of locked nucleic acids (LNA). *J. Mol. Recognit.* **2000**, *13*, 44-53.
11. Obika, S.; Nanbu, D.; Hari, Y.; Andoh, J.; Morio, K.; Doi, T.; Imanishi, T. Stability and structural features of the duplexes containing nucleoside analogues with a fixed N-type conformation, 2'-O,4'-C-methylenribonucleosides. *Tetrahedron Lett.* **1998**, *39*, 5401-5404.
12. Wengel, J. Synthesis of 3'-C- and 4'-C-branched oligodeoxynucleotides and the development of locked nucleic acid (LNA). *Acc. Chem. Res.* **1999**, *32*, 301-310.
13. Braasch, D.A.; Corey, D.R. Locked nucleic acid (LNA): Fine-tuning the recognition of DNA and RNA. *Chem. Biol.* **2001**, *8*, 1-7.
14. Kurreck, J.; Wyszko, E.; Gillen, C.; Erdmann, V.A. Design of antisense oligonucleotides stabilized by locked nucleic acids. *Nucleic Acids Res.* **2002**, *30*, 1911-1918.
15. Bondensgaard, K.; Petersen, M.; Singh, S.K.; Rajwanshi, V.K.; Kumar, R.; Wengel, J.; Jacobsen, J.P. Structural studies of LNA:RNA duplexes by NMR: Conformations and implications for RNase H activity. *Chemistry* **2000**, *6*, 2687-2695.
16. Jepsen, J.S.; Wengel, J. LNA-antisense rivals siRNA for gene silencing. *Curr. Opin. Drug Discov. Devel.* **2004**, *7*, 188-194.

17. Roberts, J.; Palma, E.; Sazani, P.; Orum, H.; Cho, M.; Kole, R. Efficient and persistent splice switching by systemically delivered LNA oligonucleotides in mice. *Mol. Ther.* **2006**, *14*, 471-475.
18. Graziewicz, M.A.; Tarrant, T.K.; Buckley, B.; Roberts, J.; Fulton, L.; Hansen, H.; Orum, H.; Kole, R.; Sazani, P. An endogenous TNF-alpha antagonist induced by splice-switching oligonucleotides reduces inflammation in hepatitis and arthritis mouse models. *Mol. Ther.* **2008**, *16*, 1316-1322.
19. Guterstam, P.; Lindgren, M.; Johansson, H.; Tedebark, U.; Wengel, J.; El Andaloussi, S.; Langel, U. Splice-switching efficiency and specificity for oligonucleotides with locked nucleic acid monomers. *Biochem. J.* **2008**, *412*, 307-313.
20. Childs, J.L.; Disney, M.D.; Turner, D.H. Oligonucleotide directed misfolding of RNA inhibits *Candida albicans* group I intron splicing. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 11091-11096.
21. Fluiter, K.; Mook, O.R.; Baas, F. The therapeutic potential of LNA-modified siRNAs: Reduction of off-target effects by chemical modification of the siRNA sequence. *Methods Mol. Biol.* **2009**, *487*, 189-203.
22. Wang, L.; Yang, C.J.; Medley, C.D.; Benner, S.A.; Tan, W. Locked nucleic acid molecular beacons. *J. Am. Chem. Soc.* **2005**, *127*, 15664-15665.
23. Buh Gasparic, M.; Tengs, T.; La Paz, J.L.; Holst-Jensen, A.; Pla, M.; Esteve, T.; Zel, J.; Gruden, K. Comparison of nine different real-time PCR chemistries for qualitative and quantitative applications in GMO detection. *Anal. Bioanal. Chem.* **2010**, *396*, 2023-2029.
24. Martinez, K.; Estevez, M.C.; Wu, Y.; Phillips, J.A.; Medley, C.D.; Tan, W. Locked nucleic acid based beacons for surface interaction studies and biosensor development. *Anal. Chem.* **2009**, *81*, 3448-3454.
25. Morandi, L.; Ferrari, D.; Lombardo, C.; Pession, A.; Tallini, G. Monitoring HCV RNA viral load by locked nucleic acid molecular beacons real time PCR. *J. Virol. Methods* **2007**, *140*, 148-154.
26. Stenvang, J.; Silahatoglu, A.N.; Lindow, M.; Elmen, J.; Kauppinen, S. The utility of LNA in microRNA-based cancer diagnostics and therapeutics. *Semin. Cancer Biol.* **2008**, *18*, 89-102.
27. Vester, B.; Lundberg, L.B.; Sorensen, M.D.; Babu, B.R.; Douthwaite, S.; Wengel, J. LNAzymes: Incorporation of LNA-type monomers into DNAzymes markedly increases RNA cleavage. *J. Am. Chem. Soc.* **2002**, *124*, 13682-13683.
28. Donini, S.; Clerici, M.; Wengel, J.; Vester, B.; Peracchi, A. The advantages of being locked. ASSESSING the cleavage of short and long RNAs by locked nucleic acid-containing 8–17 deoxyribozymes. *J. Biol. Chem.* **2007**, *282*, 35510-35518.
29. Fluiter, K.; Frieden, M.; Vreijling, J.; Koch, T.; Baas, F. Evaluation of LNA-modified DNAzymes targeting a single nucleotide polymorphism in the large subunit of RNA polymerase II. *Oligonucleotides* **2005**, *15*, 246-254.
30. Fahmy, R.G.; Khachigian, L.M. Locked nucleic acid modified DNA enzymes targeting early growth response-1 inhibit human vascular smooth muscle cell growth. *Nucleic Acids Res.* **2004**, *32*, 2281-2285.
31. Jakobsen, M.R.; Haasnoot, J.; Wengel, J.; Berkhout, B.; Kijms, J. Efficient inhibition of HIV-1 expression by LNA modified antisense oligonucleotides and DNAzymes targeted to functionally selected binding sites. *Retrovirology* **2007**, *4*, 29.

32. Kaur, H.; Scaria, V.; Maiti, S. "Locked onto the Target": Increasing the efficiency of antagomirzymes using locked nucleic acid modifications. *Biochemistry* **2010**, *49*, 9449-9456.
33. Schubert, S.; Furste, J.P.; Werk, D.; Grunert, H.P.; Zeichhardt, H.; Erdmann, V.A.; Kurreck, J. Gaining target access for deoxyribozymes. *J. Mol. Biol.* **2004**, *339*, 355-363.
34. Vester, B.; Hansen, L.H.; Lundberg, L.B.; Babu, B.R.; Sorensen, M.D.; Wengel, J.; Douthwaite, S. Locked nucleoside analogues expand the potential of DNAzymes to cleave structured RNA targets. *BMC Mol. Biol.* **2006**, *7*, 19.
35. Vester, B.; Lundberg, L.B.; Sorensen, M.D.; Babu, B.R.; Douthwaite, S.; Wengel, J. Improved RNA cleavage by LNAzyme derivatives of DNAzymes. *Biochem. Soc. Trans.* **2004**, *32*, 37-40.
36. Schubert, S.; Gul, D.C.; Grunert, H.P.; Zeichhardt, H.; Erdmann, V.A.; Kurreck, J. RNA cleaving '10-23' DNAzymes with enhanced stability and activity. *Nucleic Acids Res.* **2003**, *31*, 5982-5992.
37. Abdelgany, A.; Uddin, M.K.; Wood, M.; Taira, K.; Beeson, D. Design of efficient DNAzymes against muscle AChR alpha-subunit cRNA *in vitro* and in HEK 293 cells. *J. RNAi Gene Silencing* **2005**, *1*, 88-96.
38. Wahlestedt, C.; Salmi, P.; Good, L.; Kela, J.; Johnsson, T.; Hokfelt, T.; Broberger, C.; Porreca, F.; Lai, J.; Ren, K.; *et al.* Potent and nontoxic antisense oligonucleotides containing locked nucleic acids. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 5633-5638.
39. Straarup, E.M.; Fisker, N.; Hedtjarn, M.; Lindholm, M.W.; Rosenbohm, C.; Aarup, V.; Hansen, H.F.; Orum, H.; Hansen, J.B.; Koch, T. Short locked nucleic acid antisense oligonucleotides potently reduce apolipoprotein B mRNA and serum cholesterol in mice and non-human primates. *Nucleic Acids Res.* **2010**, *38*, 7100-7111.
40. Fluiter, K.; ten Asbroek, A.L.; de Wissel, M.B.; Jakobs, M.E.; Wissenbach, M.; Olsson, H.; Olsen, O.; Oerum, H.; Baas, F. *In vivo* tumor growth inhibition and biodistribution studies of locked nucleic acid (LNA) antisense oligonucleotides. *Nucleic Acids Res.* **2003**, *31*, 953-962.
41. Elmen, J.; Lindow, M.; Silahatoglu, A.; Bak, M.; Christensen, M.; Lind-Thomsen, A.; Hedtjarn, M.; Hansen, J.B.; Hansen, H.F.; Straarup, E.M.; *et al.* Antagonism of microRNA-122 in mice by systemically administered LNA-antimiR leads to up-regulation of a large set of predicted target mRNAs in the liver. *Nucleic Acids Res.* **2008**, *36*, 1153-1162.
42. Stein, C.A.; Hansen, J.B.; Lai, J.; Wu, S.; Voskresenskiy, A.; Hog, A.; Worm, J.; Hedtjarn, M.; Souleimanian, N.; Miller, P.; *et al.* Efficient gene silencing by delivery of locked nucleic acid antisense oligonucleotides, unassisted by transfection reagents. *Nucleic Acids Res.* **2010**, *38*, e3.
43. Swayze, E.E.; Siwkowski, A.M.; Wancewicz, E.V.; Migawa, M.T.; Wyrzykiewicz, T.K.; Hung, G.; Monia, B.P.; Bennett, C.F. Antisense oligonucleotides containing locked nucleic acid improve potency but cause significant hepatotoxicity in animals. *Nucleic Acids Res.* **2007**, *35*, 687-700.
44. Santaris Pharma A/S. Santaris Pharma A/S advances miravirsin, the first microRNA-targeted drug to enter clinical trials, into Phase 2 to treat patients infected with Hepatitis C virus. Available online: <http://www.santaris.com/news/2010/09/23/santaris-pharma-advances-miravirsin-first-microrna-targeted-drug-enter-clinical-tria/> (accessed on: May 16, 2011).
45. Santaris Pharma A/S. Another LNA-based RNA Inhibitor Enters Clinical Trials. Available online: <http://www.santaris.com/news/2009/03/20/another-lna-based-rna-inhibitor-enters-clinical-trials/> (accessed on: May 16, 2011).

46. Elmen, J.; Thonberg, H.; Ljungberg, K.; Frieden, M.; Westergaard, M.; Xu, Y.; Wahren, B.; Liang, Z.; Orum, H.; Koch, T.; Wahlestedt, C. Locked nucleic acid (LNA) mediated improvements in siRNA stability and functionality. *Nucleic Acids Res.* **2005**, *33*, 439-447.
47. Gao, S.; Dagnaes-Hansen, F.; Nielsen, E.J.; Wengel, J.; Besenbacher, F.; Howard, K.A.; Kjems, J. The effect of chemical modification and nanoparticle formulation on stability and biodistribution of siRNA in mice. *Mol. Ther.* **2009**, *17*, 1225-1233.
48. Mook, O.R.; Baas, F.; de Wissel, M.B.; Fluiters, K. Evaluation of locked nucleic acid-modified small interfering RNA *in vitro* and *in vivo*. *Mol. Cancer Ther.* **2007**, *6*, 833-843.
49. Frieden, M.; Christensen, S.M.; Mikkelsen, N.D.; Rosenbohm, C.; Thru, C.A.; Westergaard, M.; Hansen, H.F.; Orum, H.; Koch, T. Expanding the design horizon of antisense oligonucleotides with alpha-L-LNA. *Nucleic Acids Res.* **2003**, *31*, 6365-6372.
50. Nagahama, K.; Veedu, R.N.; Wengel, J. Nuclease resistant methylphosphonate-DNA/LNA chimeric oligonucleotides. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2707-2709.
51. Morita, K.; Takagi, M.; Hasegawa, C.; Kaneko, M.; Tsutsumi, S.; Sone, J.; Ishikawa, T.; Imanishi, T.; Koizumi, M. Synthesis and properties of 2'-O,4'-C-ethylene-bridged nucleic acids (ENA) as effective antisense oligonucleotides. *Bioorg. Med. Chem.* **2003**, *11*, 2211-2226.
52. Crinelli, R.; Bianchi, M.; Gentilini, L.; Magnani, M. Design and characterization of decoy oligonucleotides containing locked nucleic acids. *Nucleic Acids Res.* **2002**, *30*, 2435-2443.
53. Stoltenburg, R.; Reinemann, C.; Strehlitz, B. SELEX-A (r)evolutionary method to generate high-affinity nucleic acid ligands. *Biomol. Eng.* **2007**, *4*, 381-403.
54. Bunka, D.H.; Stockley, P.G. Aptamers come of age—At last. *Nat. Rev. Microbiol.* **2006**, *4*, 588-596.
55. Syed, M.A.; Pervaiz, S. Advances in aptamers. *Oligonucleotides* **2010**, *20*, 215-224.
56. Cho, E.J.; Lee, J.W.; Ellington, A.D. Applications of aptamers as sensors. *Annu. Rev. Anal. Chem. (Palo Alto Calif)* **2009**, *2*, 241-264.
57. Duconge, F.; Toulme, J.J. *In vitro* selection identifies key determinants for loop-loop interactions: RNA aptamers selective for the TAR RNA element of HIV-1. *RNA* **1999**, *5*, 1605-1614.
58. Darfeuille, F.; Hansen, J.B.; Orum, H.; Di Primo, C.; Toulme, J.J. LNA/DNA chimeric oligomers mimic RNA aptamers targeted to the TAR RNA element of HIV-1. *Nucleic Acids Res.* **2004**, *32*, 3101-3107.
59. Di Primo, C.; Rudloff, I.; Reigadas, S.; Arzumanov, A.A.; Gait, M.J.; Toulme, J.J. Systematic screening of LNA/2'-O-methyl chimeric derivatives of a TAR RNA aptamer. *FEBS Lett.* **2007**, *581*, 771-774.
60. Hicke, B.J.; Marion, C.; Chang, Y.F.; Gould, T.; Lynott, C.K.; Parma, D.; Schmidt, P.G.; Warren, S. Tenascin-C aptamers are generated using tumor cells and purified protein. *J. Biol. Chem.* **2001**, *276*, 48644-48654.
61. Schmidt, K.S.; Borkowski, S.; Kurreck, J.; Stephens, A.W.; Bald, R.; Hecht, M.; Friebe, M.; Dinkelborg, L.; Erdmann, V.A. Application of locked nucleic acids to improve aptamer *in vivo* stability and targeting function. *Nucleic Acids Res.* **2004**, *32*, 5757-5765.
62. Kasahara, Y.; Kitadume, S.; Morihira, K.; Kuwahara, M.; Ozaki, H.; Sawai, H.; Imanishi, T.; Obika, S. Effect of 3'-end capping of aptamer with various 2',4'-bridged nucleotides: Enzymatic post-modification toward a practical use of polyclonal aptamers. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1626-1629.

63. Hernandez, F.J.; Kalra, N.; Wengel, J.; Vester, B. Aptamers as a model for functional evaluation of LNA and 2'-amino LNA. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6585-6587.
64. Shangguan, D.; Tang, Z.; Mallikaratchy, P.; Xiao, Z.; Tan, W. Optimization and modifications of aptamers selected from live cancer cell lines. *Chembiochem* **2007**, *8*, 603-606.
65. Mallikaratchy, P.R.; Ruggiero, A.; Gardner, J.R.; Kuryavyi, V.; Maguire, W.F.; Heaney, M.L.; McDevitt, M.R.; Patel, D.J.; Scheinberg, D.A. A multivalent DNA aptamer specific for the B-cell receptor on human lymphoma and leukemia. *Nucleic Acids Res.* **2010**, *39*, 2458-2469.
66. Pasternak, A.; Hernandez, F.J.; Rasmussen, L.M.; Vester, B.; Wengel, J. Improved thrombin binding aptamer by incorporation of a single unlocked nucleic acid monomer. *Nucleic Acids Res.* **2011**, *39*, 1155-1164.
67. Bock, L.C.; Griffin, L.C.; Latham, J.A.; Vermaas, E.H.; Toole, J.J. Selection of single-stranded DNA molecules that bind and inhibit human thrombin. *Nature* **1992**, *355*, 564-566.
68. Virno, A.; Randazzo, A.; Giancola, C.; Bucci, M.; Cirino, G.; Mayol, L. A novel thrombin binding aptamer containing a G-LNA residue. *Bioorg. Med. Chem.* **2007**, *15*, 5710-5718.
69. Bonifacio, L.; Church, F.C.; Jarstfer, M.B. Effect of locked-nucleic acid on a biologically active g-quadruplex. A structure-activity relationship of the thrombin aptamer. *Int. J. Mol. Sci.* **2008**, *9*, 422-433.
70. Tasset, D.M.; Kubik, M.F.; Steiner, W. Oligonucleotide inhibitors of human thrombin that bind distinct epitopes. *J. Mol. Biol.* **1997**, *272*, 688-698.
71. Hernandez, F.J.; Dondapati, S.K.; Ozalp, V.C.; Pinto, A.; O'Sullivan, C.K.; Klar, T.A.; Katakis, I. Label free optical sensor for Avidin based on single gold nanoparticles functionalized with aptamers. *J. Biophotonics* **2009**, *2*, 227-231.
72. Mallikaratchy, P.; Tang, Z.; Kwame, S.; Meng, L.; Shangguan, D.; Tan, W. Aptamer directly evolved from live cells recognizes membrane bound immunoglobulin heavy mu chain in Burkitt's lymphoma cells. *Mol. Cell. Proteomics* **2007**, *6*, 2230-2238.
73. Tang, Z.; Shangguan, D.; Wang, K.; Shi, H.; Sefah, K.; Mallikaratchy, P.; Chen, H.W.; Li, Y.; Tan, W. Selection of aptamers for molecular recognition and characterization of cancer cells. *Anal. Chem.* **2007**, *79*, 4900-4907.
74. Zaug, A.J.; Cech, T.R., *In vitro* splicing of the ribosomal RNA precursor in nuclei of *Tetrahymena*. *Cell* **1980**, *19*, 331-338.
75. Kruger, K.; Grabowski, P.J.; Zaug, A.J.; Sands, J.; Gottschling, D.E.; Cech, T.R. Self-splicing RNA: Autoexcision and autocyclization of the ribosomal RNA intervening sequence of *Tetrahymena*. *Cell* **1982**, *31*, 147-157.
76. Weigand, B.-S.; Zerressen, A.; Schlatterer, J.C.; Helm, M.; Jaschke, A. *In vitro* selection of short, catalytically active oligonucleotides; In *The Aptamer Handbook: Functional Oligonucleotides and Their Applications*, Klussmann, S., Ed.; Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany, 2006; Volume 1, pp. 211-227.
77. Pan, W.; Clawson, G.A. Catalytic DNAzymes: Derivations and functions. *Expert Opin. Biol. Ther.* **2008**, *8*, 1071-1085.
78. Santoro, S.W.; Joyce, G.F. A general purpose RNA-cleaving DNA enzyme. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 4262-4266.

79. Robaldo, L.; Montserrat, J.M.; Iribarren, A.M. 10-23 DNAzyme modified with (2'*R*)- and (2'*S*)-2'-deoxy-2'-C-methyluridine in the catalytic core. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4367-4370.
80. Lobedanz, S.; Vester, B. University of Southern Denmark, Campusvej 55 Odense M 5230, Denmark, 2011
81. Christiansen, J.K.; Lobedanz, S.; Arar, K.; Wengel, J.; Vester, B. LNA nucleotides improve cleavage efficiency of singular and binary hammerhead ribozymes. *Bioorg. Med. Chem.* **2007**, *15*, 6135-6143.
82. Persson, T.; Hartmann, R.K.; Eckstein, F. Selection of hammerhead ribozyme variants with low Mg²⁺ requirement: Importance of stem-loop II. *ChemBiochem* **2002**, *3*, 1066-1071.
83. Fedoruk-Wyszomirska, A.; Szymanski, M.; Wyszko, E.; Barciszewska, M.Z.; Barciszewski, J. Highly active low magnesium hammerhead ribozyme. *J. Biochem.* **2009**, *145*, 451-459.
84. Pan, T.; Uhlenbeck, O.C. A small metalloribozyme with a two-step mechanism. *Nature* **1992**, *358*, 560-563.
85. Pan, T.; Dichtl, B.; Uhlenbeck, O.C. Properties of an *in vitro* selected Pb²⁺ cleavage motif. *Biochemistry* **1994**, *33*, 9561-9565.
86. Julien, K.R.; Sumita, M.; Chen, P.H.; Laird-Offringa, I.A.; Hoogstraten, C.G. Conformationally restricted nucleotides as a probe of structure-function relationships in RNA. *RNA* **2008**, *14*, 1632-1643.
87. Pasternak, A.; Wengel, J. Unlocked nucleic acid—An RNA modification with broad potential. *Org. Biomol. Chem.* **2011**, *9*, 3591-3597.
88. Jensen, T.B.; Langkjaer, N.; Wengel, J. Unlocked nucleic acid (UNA) and UNA derivatives: Thermal denaturation studies. *Nucleic Acids Symp. Ser. (Oxf)* **2008**, 133-134.
89. Itkes, A.V.; Karpeisky, M.; Kartasheva, O.N.; Mikhailov, S.N.; Moiseyev, G.P.; Pfeleiderer, W.; Charubala, R.; Yakovlev, G.I. A route to 2',5'-oligoadenylates with increased stability towards phosphodiesterases. *FEBS Lett.* **1988**, *236*, 325-328.
90. Bramsen, J.B.; Laursen, M.B.; Nielsen, A.F.; Hansen, T.B.; Bus, C.; Langkjaer, N.; Babu, B.R.; Hojland, T.; Abramov, M.; Van Aerschot, A.; *et al.* A large-scale chemical modification screen identifies design rules to generate siRNAs with high activity, high stability and low toxicity. *Nucleic Acids Res.* **2009**, *37*, 2867-2881.
91. Laursen, M.B.; Pakula, M.M.; Gao, S.; Fluiters, K.; Mook, O.R.; Baas, F.; Langkjaer, N.; Wengel, S.L.; Wengel, J.; Kjems, J.; *et al.* Utilization of unlocked nucleic acid (UNA) to enhance siRNA performance *in vitro* and *in vivo*. *Mol. Biosyst.* **2010**, *6*, 862-870.
92. Bramsen, J.B.; Pakula, M.M.; Hansen, T.B.; Bus, C.; Langkjaer, N.; Odadzic, D.; Smicius, R.; Wengel, S.L.; Chattopadhyaya, J.; Engels, J.W.; *et al.* A screen of chemical modifications identifies position-specific modification by UNA to most potently reduce siRNA off-target effects. *Nucleic Acids Res.* **2010**, *38*, 5761-5773.
93. Padmanabhan, K.; Padmanabhan, K.P.; Ferrara, J.D.; Sadler, J.E.; Tulinsky, A. The structure of alpha-thrombin inhibited by a 15-mer single-stranded DNA aptamer. *J. Biol. Chem.* **1993**, *268*, 17651-17654.
94. Macaya, R.F.; Schultze, P.; Smith, F.W.; Roe, J.A.; Feigon, J. Thrombin-binding DNA aptamer forms a unimolecular quadruplex structure in solution. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 3745-3749.

95. Kelly, J.A.; Feigon, J.; Yeates, T.O. Reconciliation of the X-ray and NMR structures of the thrombin-binding aptamer d(GGTTGGTGTGGTTGG). *J. Mol. Biol.* **1996**, *256*, 417-422.
96. Tuerk, C.; Gold, L. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. *Science* **1990**, *249*, 505-510.
97. Ellington, A.D.; Szostak, J.W. *In vitro* selection of RNA molecules that bind specific ligands. *Nature* **1990**, *346*, 818-822.
98. Wilson, D.S.; Szostak, J.W. *In vitro* selection of functional nucleic acids. *Annu. Rev. Biochem.* **1999**, *68*, 611-647.
99. Stoltenburg, R.; Reinemann, C.; Strehlitz, B. SELEX—A (r)evolutionary method to generate high-affinity nucleic acid ligands. *Biomol. Eng.* **2007**, *24*, 381-403.
100. Keefe, A.D.; Cload, S.T. SELEX with modified nucleotides. *Curr. Opin. Chem. Biol.* **2008**, *12*, 448-456.
101. Koshkin, A.A.; Fensholdt, J.; Pfundheller, H.M.; Lomholt, C. A simplified and efficient route to 2'-O, 4'-C-methylene-linked bicyclic ribonucleosides (locked nucleic acid). *J. Org. Chem.* **2001**, *66*, 8504-8512.
102. Madsen, A.S.; Kumar, T.S.; Wengel, J. LNA 5'-phosphoramidites for 5'→3'-oligonucleotide synthesis. *Org. Biomol. Chem.* **2010**, *8*, 5012-5016.
103. Barciszewski, J.; Medgaard, M.; Koch, T.; Kurreck, J.; Erdmann, V.A. Locked nucleic acid aptamers. *Methods Mol. Biol.* **2009**, *535*, 165-186.
104. Kuwahara, M.; Obika, S.; Nagashima, J.; Ohta, Y.; Suto, Y.; Ozaki, H.; Sawai, H.; Imanishi, T. Systematic analysis of enzymatic DNA polymerization using oligo-DNA templates and triphosphate analogs involving 2',4'-bridged nucleosides. *Nucleic Acids Res.* **2008**, *36*, 4257-4265.
105. Veedu, R.N.; Vester, B.; Wengel, J. Enzymatic incorporation of LNA nucleotides into DNA strands. *Chembiochem* **2007**, *8*, 490-492.
106. Veedu, R.N.; Vester, B.; Wengel, J. *In vitro* incorporation of LNA nucleotides. *Nucleos. Nucleot. Nucl.* **2007**, *26*, 1207-1210.
107. Veedu, R.N.; Vester, B.; Wengel, J. Polymerase chain reaction and transcription using locked nucleic acid nucleotide triphosphates. *J. Am. Chem. Soc.* **2008**, *130*, 8124-8125.
108. Veedu, R.N.; Vester, B.; Wengel, J. Novel applications of locked nucleic acids. *Nucleic Acids Symp. Ser. (Oxf)* **2007**, 29-30.
109. Veedu, R.N.; Vester, B.; Wengel, J. Efficient enzymatic synthesis of LNA-modified DNA duplexes using KOD DNA polymerase. *Org. Biomol. Chem.* **2009**, *7*, 1404-1409.
110. Veedu, R.N.; Vester, B.; Wengel, J. Polymerase directed incorporation studies of LNA-G nucleoside 5'-triphosphate and primer extension involving all four LNA nucleotides. *New J. Chem.* **2010**, *34*, 877-879.
111. Drabovich, A.; Berezovski, M.; Krylov, S.N. Selection of smart aptamers by equilibrium capillary electrophoresis of equilibrium mixtures (ECEEM). *J. Am. Chem. Soc.* **2005**, *127*, 11224-11225.
112. Berezovski, M.; Musheev, M.; Drabovich, A.; Krylov, S.N. Non-SELEX selection of aptamers. *J. Am. Chem. Soc.* **2006**, *128*, 1410-1411.
113. Mendonsa, S.D.; Bowser, M.T. *In vitro* selection of aptamers with affinity for neuropeptide Y using capillary electrophoresis. *J. Am. Chem. Soc.* **2005**, *127*, 9382-9383.

114. Mendonsa, S.D.; Bowser, M.T. *In vitro* evolution of functional DNA using capillary electrophoresis. *J. Am. Chem. Soc.* **2004**, *126*, 20-21.
115. Mosing, R.K.; Mendonsa, S.D.; Bowser, M.T. Capillary electrophoresis-SELEX selection of aptamers with affinity for HIV-1 reverse transcriptase. *Anal. Chem.* **2005**, *77*, 6107-6112.
116. Mendonsa, S.D.; Bowser, M.T. *In vitro* selection of high-affinity DNA ligands for human IgE using capillary electrophoresis. *Anal. Chem.* **2004**, *76*, 5387-5392.
117. Berezovski, M.; Drabovich, A.; Krylova, S.M.; Musheev, M.; Okhonin, V.; Petrov, A.; Krylov, S.N. Nonequilibrium capillary electrophoresis of equilibrium mixtures: A universal tool for development of aptamers. *J. Am. Chem. Soc.* **2005**, *127*, 3165-3171.
118. Berezovski, M.V.; Musheev, M.U.; Drabovich, A.P.; Jitkova, J.V.; Krylov, S.N. Non-SELEX: Selection of aptamers without intermediate amplification of candidate oligonucleotides. *Nat. Protoc.* **2006**, *1*, 1359-1369.
119. Yang, X.; Bassett, S.E.; Li, X.; Luxon, B.A.; Herzog, N.K.; Shope, R.E.; Aronson, J.; Prow, T.W.; Leary, J.F.; Kirby, R.; *et al.* Construction and selection of bead-bound combinatorial oligonucleoside phosphorothioate and phosphorodithioate aptamer libraries designed for rapid PCR-based sequencing. *Nucleic Acids Res.* **2002**, *30*, e132.
120. Katilius, E.; Flores, C.; Woodbury, N.W. Exploring the sequence space of a DNA aptamer using microarrays. *Nucleic Acids Res.* **2007**, *35*, 7626-7635.
121. Knight, C.G.; Platt, M.; Rowe, W.; Wedge, D.C.; Khan, F.; Day, P.J.; McShea, A.; Knowles, J.; Kell, D.B. Array-based evolution of DNA aptamers allows modelling of an explicit sequence-fitness landscape. *Nucleic Acids Res.* **2009**, *37*, e6.
122. Platt, M.; Rowe, W.; Wedge, D.C.; Kell, D.B.; Knowles, J.; Day, P.J. Aptamer evolution for array-based diagnostics. *Anal. Biochem.* **2009**, *390*, 203-205.
123. Rowe, W.; Platt, M.; Wedge, D.C.; Day, P.J.; Kell, D.B.; Knowles, J.D. Convergent evolution to an aptamer observed in small populations on DNA microarrays. *Phys. Biol.* **2010**, *7*, 036007.
124. Singh, S.K.; Kumar, R.; Wengel, J. Synthesis of 2'-amino-LNA: A novel conformationally restricted high-affinity oligonucleotide analogue with a handle. *J. Org. Chem.* **1998**, *63*, 10035-10039.