Published in final edited form as:
Curr Pharm Des. 2012 ; 18(14): 1984–1991.

Targeting DNA G-Quadruplex Structures with Peptide Nucleic Acids

Igor G. Panyutin[1,*], **Mykola I. Onyshchenko**[1,3], **Ethan A. Englund**[2], **Daniel H. Appella**[2], and **Ronald D. Neumann**[1]

[1]Departmeut of Radiology and Imaging Sciences, Clinical Center, National Institutes of Health, Bethesda, MD 20892, USA

[2]Laboratory of Bioorganic Chemistry, NIDDK, National Institutes of Health, Bethesda, MD 20892, USA

[3]Imaging Sciences Training Program, Clinical Center and National Institute of Biomedical Imaging and Bioengineering, National Institutes of Health, Bethesda, MD 20892, USA

Abstract

Regulation of genetic functions based on targeting DNA or RNA sequences with complementary oligonucleotides is especially attractive in the post-genome era. Oligonucleotides can be rationally designed to bind their targets based on simple nucleic acid base pairing rules. However, the use of natural DNA and RNA oligonucleotides as targeting probes can cause numerous off-target effects. In addition, natural nucleic acids are prone to degradation *in vivo* by various nucleases. To address these problems, nucleic acid mimics such as peptide nucleic acids (PNA) have been developed. They are more stable, show less off-target effects, and, in general, have better binding affinity to their targets. However, their high affinity to DNA can reduce their sequence-specificity. The formation of alternative DNA secondary structures, such as the G-quadruplex, provides an extra level of specificity as targets for PNA oligomers. PNA probes can target the loops of G-quadruplex, invade the core by forming PNA-DNA guanine-tetrads, or bind to the open bases on the complementary cytosine-rich strand. Not only could the development of such G-quadruplex-specific probes allow regulation of gene expression, but it will also provide a means to clarify the biological roles G-quadruplex structures may possess.

Keywords

Peptide Nucleic Acids; G-quadruplex; gene expression regulation

Introduction

The human genome is enriched with sequences that can potentially form G-quadruplex structures [1, 2]. Usually, such sequences consist of four runs of three or more guanines separated by short linker sequences. The runs of guanines form the core of the quadruplex stabilized by guanine tetrads while the linker sequences form loops of various conformations [3]. Human telomeric repeats contain the greatest number of such sequences [4]. In addition, numerous genes possess sequences capable of forming G-quadruplex structures in their regulatory regions [5, 6]. Proto-oncogenes are highly enriched with such G-quadruplex-forming sequences compared to tumor suppressor genes [7]. Furthermore, there is mounting

[*]Corresponding author: Igor G. Panyutin, NIH/CC/RAD&IS, Bldg. 10, Rm. 1C401, Bethesda, MD 2089180 USA, igorp@helix.nih.gov, Tel. (301) 496-8308, Fax. (301) 480-9712.

evidence that G-quadruplex structures may play roles not only in telomere maintenance but in regulation of gene expression as well [8]. Numerous small molecules with higher affinity to G-quadruplex versus duplex DNA have been synthesized [9, 10]; some of these are potent inhibitors of the telomere-extending enzyme, telomerase, which is overexpressed in cancer cells, and are being extensively tested as anticancer drugs [11]. Targeting G-quadruplex structures in the intrachromosomal regulatory regions is more challenging because of the wide variety of G-quadruplex structures that can form in different genes based on their unique sequences [8]. Even though molecules have been designed to specifically bind to the G-quadruplex structures formed in particular genes, the task remains very challenging.

An alternative approach to targeting specific G-quadruplex structures is based on using short oligonucleotides that bind to the complementary DNA bases that become accessible after G-quadruplex formation. This approach is similar to antisense [12], antigene [13], and RNAi-based [14] approaches, and is based on simple nucleic acids complementarity rules. While developing antisense, antigene and RNAi-based therapies, it became clear that for this approach to be effective, chemically modified analogs or nucleic acid mimics should be used. One such class of molecules, peptide nucleic acids (PNAs), are nucleic acid mimics in which the natural nucleobases are connected to an achiral, uncharged polyamide backbone [15] (Figure 1). PNA oligomers form very stable duplexes with complementary nucleic acids; they can even invade double-stranded DNA under certain conditions. For example, when the DNA is negatively supercoiled, PNA and DNA will complex and form a P-loop [15, 16] (Figure 2). In addition, guanine-rich PNA are able to form G-quadruplexes by themselves or DNA-PNA hybrid G-quadruplex structures [17].

Based on these unique properties, several approaches to target G-quadruplexes with PNA have been proposed. First, guanine-rich PNA can invade a DNA G-quadruplex and simultaneously bind to the complementary cytosine-rich strand, thus facilitating G-quadruplex formation [18, 19]. Second, short PNA oliomgers can be designed to bind to the single-stranded DNA (ssDNA) in the exposed loops of G-quadruplex structures [20]. Third, PNA can bind exclusively to the complementary cytosine-strand, thus facilitating G-quadruplex extrusion in the G-rich strand without interfering with native DNA G-quadruplex conformation [21] (Figure 3).

In this short review, we will describe all these approaches. We also will provide background on PNA chemistry and design, and speculate on possible mechanisms of PNA-based strategies to regulate gene expression by targeting a G-quadruplex.

PNA as DNA sequence-specific targeting drugs

There are many approaches to make gene-specific drugs, but a system that takes advantage of nucleobase hydrogen bond recognition would be of general use for any gene target (Figure 4). Nucleic acid derivatives are attractive drug candidates over natural nucleic acids. PNAs are non-natural nucleic acids in which the natural nucleobases are preserved but appended to an uncharged, achiral pseudo-peptide backbone in lieu of the natural sugar phosphate backbone [22]. Because the PNA oligomers are uncharged, they tend to exhibit tighter binding to natural nucleic acids due to the lack of poly anionic charge-charge interactions [23]. Furthermore, PNA oligomers are resistant to degradation *in vivo* because they are not recognized by nucleases or proteases, and their stability makes them attractive candidates for antigene, antisense, or nucleic acid probes [24].

Chemical modification of PNA oligomers can abrogate some of its inherent shortcomings, such as solubility, cell permeability, or bioavailability (Figure 5). The use of modified PNA residues in PNA oligomers can also affect the binding affinity and selectivity to nucleic acids through backbone rigidification and preorganization [25–27], increase solubility [28],

provide a handle for further conjugation or ligand display [29] or increase cellular uptake [30]. Recent work with diethylene glycol γ-substituted PNA residues afforded oligomers that had both higher binding affinity to DNA and increased aqueous solubility [31]. However, the thermodynamic data indicated that preorganization might not be the reason for the increased binding affinity in this example.

PNA oligomers targeting genomic DNA in a sequence specific manner has already seen remarkable progress [32–36]. When cell-penetrating peptides are conjugated to an antigene PNA oligomer, inhibition of gene expression has been demonstrated [37–39]. Using pseudovirion delivery agents as another means of delivering PNA oligomers into cells has similarly shown promise in suppressing gene expression associated with drug resistant cancer cells [40]. Besides targeted gene inhibition, another application of PNA oligomers involves promoting the repair and recovery of gene function [41, 42]. All of these approaches utilize the nucleic acid sequence specificity rather than recognition of nucleic acid secondary structure [43].

G-quadruplex and other alternative DNA structures

DNA forms a remarkable variety of secondary structures beyond the well-known B-form double helix [44]. Examples include A-form DNA duplex [45], the left handed Z-DNA [46], triplexes formed from binding the Hoogsteen face of purine bases in the major groove (Figure 4) [47], cytosine-rich i-motif [48] and G-quadruplexes [49,50]. Many of these types of nucleic acid structures depend on non-Watson-Crick hydrogen bonding such as wobble base pairing or sheared-type base pairing [51]. However, the question whether many of these alternative DNA structures play a biological role remains open to this day. Some alternative structures were found under the conditions that are normally not present in cells, e.g., low pH, extremely supercoiled DNA, crystallized DNA, etc. [52]. Some of these secondary structures could not form readily under physiological conditions. Nevertheless, all of these structures tend to be sequence specific and some are observed *in situ* and may play a direct role in biological processes. For example, DNA triplexes utilizing Hoogsteen hydrogen bond recognition have been linked to several disease states [53]. This type of secondary structure only forms in stretches of polypurine/polypyrimidine tracks of DNA where another pyrimidine rich strand can bind the Hoogsteen face of the polypurine strand (Figure 3). The tips of vertebrate chromosomes, telomeres, contain multiple GGGTTA repeats of single stranded DNA which can form G-quadruplexes and play an integral role in preventing chromosomal deterioration or unwanted chromosomal fusion [54]. The sequences potentially forming G-quadruplex DNA structures are highly abundant along the human genome, especially in regulatory regions [55]. This observation might indirectly indicate the possible epigenetic role of these DNA structures.

PNA oligomers also have the potential to form many secondary structures, either with itself or in combination with natural nucleic acids. Nielsen and coworkers originally designed PNA to bind to the major groove (i.e. the Hoogsteen hydrogen bonding mode) of DNA duplexes [56]. It was only afterwards they discovered that PNA oligomers invade DNA duplexes and form stable $(PNA)_2$:DNA triplexes. Today, PNA:DNA triplexes are well-known, and Bis-PNAs (PNA oligomers designed to bind polypurine DNA in both Watson-Crick and Hoogsteen hydrogen bonding modes connected through a flexible linker) invade even long tracks of duplex DNA to form very stable triplex structures [57–59] (Figure 2). PNA oligomers also form stable quadruplexes in the presence of appropriate cations (sodium, potassium, ammonium, etc.) [60]. Guanine-rich PNA oliogmers form stable heterocomplexes with guanine-rich DNA oligomers [61]. The stability and selectivity of PNA quadruplexes can be modified by using PNA derivatives. The use of γ-substituted PNA can bias PNA oligomers towards binding in the guanine-quadruplex mode versus

duplex binding [18]. Furthermore, the selective arrangement of *trans*-cyclopentane PNA residues can increase the stability of PNA:DNA heterocomplexes while discouraging the competing PNA quadruplex formation [62]. The range and diversity of secondary structures that PNA forms or recognizes shows that trying to increase selectivity through targeting non-duplex complexes could be a worthwhile strategy.

Modes of targeting DNA quadruplexes with PNAs

PNAs were designed to target G-rich, C-rich, and/or both DNA strands of a G-quadruplex. Initially, PNA probes containing guanines were designed to invade homologous DNA G-quadruplex-forming sequences and participate in the formation of heterogeneous DNA-PNA guanine tetrads. This idea was first put forward by Armitage and co-workers who noticed that guanine-rich PNA formed hetero-quadruplexes with homologous DNA oligomers [17, 61]. This approach is based on targeting the guanine-rich quadruplex-forming DNA strand [17–19, 61, 63–66]. Instead of G-quadruplex invasion, guanine rich PNA probes can alternatively bind to the complementary cytosine-rich DNA strand, thus providing additional stabilization of the G-quadruplex-PNA complex. DNA sequences with four guanine runs can also be targeted in this mode via formation of two consecutive quadruplexes each consisting of two PNA- and two DNA-strands [19] (Figure 3, box, bottom). To increase the efficiency and specificity of quadruplex invasion, Lusvarghi et al. utilized modified PNA probes. They incorporated abasic sites as well as chiral modifications to the backbone of PNA and showed an improvement in the selectivity of quadruplex versus duplex formation [18]. Paul and coauthors [64] proposed another model of G-quadruplex targeting with PNAs. They designed a guanine-rich PNA probe that combines with three guanine runs of a human telomere sequence to form an intermolecular PNA-DNA G-quadruplex in a “3+1” mode. The resulting complex mimics the biologically relevant pure DNA telomeric quadruplex. However, the PNA probes described above could not invade duplex DNA even if the latter contained the motif complementary to the PNA sequence. Therefore, further efforts are required to increase the ability of PNA of invade duplex DNA to make these types of strategies viable.

An alternative approach was used by Amato et al [20] to target DNA nucleobases in the loops of G-quadruplex structure and exclude disruption of guanine tetrads within the quadruplex. They screened a small library of short PNAs complementary to a part of quadruplex-forming DNA sequence that does not contain guanines involved in G-quadruplex formation. Depending on PNA length and ionic conditions, PNAs are able to bind to the loops of the G-quadruplex and either stabilize or disrupt the quadruplex structure. In their earlier studies, Amato et al. used short cytosine-rich PNA probes to show that they could form novel G-quadruplex-PNA complexes in addition to the expected DNA-PNA heteroduplexes, depending on ionic conditions [65, 66].

Our team proposed a different approach that is based on targeting only the cytosine-rich strand complementary to the quadruplex-forming DNA strand [21, 67]. In this case, a PNA probe invades a double helix and binds the cytosine-rich strand while allowing the guanine-rich strand to form native G-quadruplex. We studied this mode of targeting using a double-stranded DNA plasmid model containing a quadruplex-forming sequence from the human *BCL2* gene promoter. Our studies on PNA invasion of duplex DNA enabled us to examine the role of quadruplex formation in PNA invasion. In addition to the plasmid with the original *BCL2* sequence, we also used a plasmid with a mutant sequence incapable of quadruplex formation but which still retained the PNAbinding sequences. Chemical probing revealed that PNA oligomers were able to invade and form a heteroduplex with the cytosine-rich strand only in plasmid DNA with the original *BCL2* sequence. We also tested the effect of total PNA charge on the efficiency of the invasion. PNAs that are positively charged or

zwitterionic (which contain both positive and negative charges but are charge neutral overall), invade the naturally supercoiled plasmid, but negatively charged PNA does not invade. Zwitterionic PNA also showed the highest specificity to the targeted sequence. Moreover, if triplex-forming bis-PNAs targeting flanking sequences were used in combination with central binding PNA (Figure 3) then invasion appeared even more favorable. Overall, we demonstrated that the potential to form a G-quadruplex facilitated PNA invasion. Our results provide strong evidence that PNA probes can be designed that are not only sequence-specific, but also quadruplex-dependent.

The aforementioned results are consistent with PNA invasion and binding facilitated by non-duplex secondary structures. Zhang *et al.* [68] demonstrated that formation of cruciform structures in palindromic regions of DNA facilitated PNA invasion. Amiard *et al.* [69] showed that multiple t-loops (lariat-like structures that form when 3′ telemeric overhang DNA invades the double-stranded telomeric repeat array) increase the ability of single-stranded DNA to invade plasmid DNA. Furthermore, invasion took place only in supercoiled DNA, implying that targeting may occur only in actively transcribed genes. As all these examples demonstrate, targeting double-stranded DNA with the potential to form alternative secondary structures has the potential to affect transcription or replication.

Possible mechanisms to regulate gene expression with G-quadruplex-specific PNA

Numerous genes possess guanine-rich sequences in promoter and other regulatory segments. Although guanine-rich regions might directly interact with DNA binding proteins, it is likely that formation of G-quadruplexes also directs interaction with proteins. In particular, the high prevalence of guanine-rich sequences in regulatory regions suggests possible regulatory roles for these quadruplex DNA structures. For example, the number of guanine-rich sequences with G-quadruplex forming potential in the human genome is almost 2 orders of magnitude higher than that in a random DNA sequence of the same length [1].

Multiple runs of guanines can be found in the promoter and enhancer regions of many genes, and particularly in oncogenes [7]. Recent studies show that these guanine-rich sequences could play a role in the regulation of gene transcription. Quadruplex formation has been studied in *PDGF-A* [70,71], *VEGF* [71–73], c-*myc* [71, 74], *KRAS* [75], *C-KIT* [76, 77], *BCL2* [78, 79] *hTERT* [80], *Rb* [81], *PDGFR*-β genes [82]. This provides an appealing opportunity for gene regulation by targeting guanine quadruplexes. While G-quadruplex formation has been demonstrated in models of single-stranded DNA oligonucleotides, several studies provide evidence of quadruplex formation in supercoiled double-stranded DNA [83] [67].

Below, we describe examples of several studies where quadruplex formation was implicated to affect gene expression. In this respect the G-quadruplex forming sequence located in the promoter region of human *C-MYC* gene [74] was one of the first described and most extensively studied. Hurley and co-workers proposed the following model of *C-MYC* expression regulation. Dynamic stress (negative supercoiling) resulting from transcription converts the duplex DNA to a G-quadruplex on the guanine-rich strand and an i-motif (proposed secondary structure in cytosine-rich sequence) on the pyrimidine-rich strand. This displaces activating transcription factors and silence gene expression. Specific proteins, namely NM23-H2 and nucleolin, that recognize the G-quadruplex are able to fold or to resolve its structure and hence regulate *C-MYC* expression [8, 84]. Authors found that inhibition of NM23-H2 silences *C-MYC* and redistribution of nucleolin from the nucleolus to the nucleoplasm negatively affects expression of this gene.

In another example, quadruplex formation in plasmid DNA during transcription *in vitro* was found using electron microscopy [85]. Authors developed a plasmid with guanine rich inserts and observed a “G-loop”, i.e. DNA/RNA hybrid on the cytosine-rich strand and a G-quadruplex on the complimentary strand during transcription. They concluded that the RNA/DNA hybrid was critical for G-quadruplex stabilization in the post-transcriptional G-loops.

Another group of authors identified a characteristic potential G-quadruplex-forming sequence element within the promoter of human thymidine kinase 1 (TK1). Their data suggested that this sequence forms an intramolecular G-quadruplex with two G-tetrads. A cell-based reporter assay revealed the role of this G-quadruplex motif in TK1 transcription. Nucleotide substitutions designed to destabilize G-quadruplex structure formation resulted in increased promoter activity, therefore, pointing on direct involvement of the G-quadruplex structure in transcription regulation of TK1. [86].

Sanders et al. described the role of Pif1 proteins (implicated in the maintenance of genome stability in yeast) in stabilizing DNA sequences that could otherwise form G-quadruplex structures by acting as a G-quadruplex resolvase. They found that for the human Pif1 to resolve a G-quadruplex, an extended (>10 nucleotide) 5′ ssDNA tail is required. The authors suggest that human Pif1 could therefore have a role in processing G4 structures that arise in the single-stranded nucleic acid intermediates formed during DNA replication and transcription. [87].

Cogoi et al. described another example of G-quadruplex-protein interaction. They found that the quadruplex-forming GA-element in the *KRAS* promoter responds to a Myc-associated zinc finger and poly(ADP-ribose) polymerase 1 proteins. Through use of an immunoprecipitation assay, they discovered that the Myc-associated zinc finger protein specifically binds to the duplex and quadruplex conformations of the GA-element, whereas poly(ADP-ribose) polymerase binds only to the G-quadruplex. Introduction of a point mutation into the quadruplex-forming sequence showed down-regulation of *KRAS,* while addition of phthalocyanines (G-quadruplex stabilizing agents) up-regulated *KRAS* expression [88].

The above examples along with a recent genome-wide study indicate that formation of G-quadruplex in the regulatory gene sequences can play a dual role in gene expression; it can cause up- or down-regulation of the gene expression [89]. In this respect, PNA probes can provide a powerful instrument to target guanine-rich regulatory DNA sequences, stabilize or destabilize G-quadruplexes, and ascertain the effect of G-quadruplex formation on the expression of a particular gene. Based on existing experimental data, several modes of gene transcription regulation through quadruplex targeting with PNA probes are possible. In one mode, PNA probes are designed to interfere with G-quadruplex recognition by protein factors. This can be achieved with PNA probes that invade G-quadruplex [17–19, 61, 63–66], or that are complementary to the loops of G-quadruplex structure [20] (Figure 6A). Alternatively, PNA oligomers complementary to the cytosine-rich strand can be used to invade DNA duplex, bind the cytosine rich strand forming a DNA:PNA duplex and allow the guanine-rich strand to form a native quadruplex structure [21, 67]. In these cases, PNA-DNA interaction leads to stabilization of native G-quadruplex structures and promotes binding of G-quadruplex specific protein factors. Once a quadruplex is stable, it can become a target for quadruplex-binding proteins. In turn, these proteins can switch on or off gene transcription, depending on that particular protein’s function (Figure 6B).

Conclusion

Targeting G-quadruplex structures with PNA has been demonstrated only *in vitro* thus far, and in many cases only models containing single-stranded guanine rich DNA are used. The next step would be to demonstrate the efficiency and specificity of this approach in cell culture models. To achieve this goal, both the affinity and specificity of PNA binding to their targets must be improved, along with methods of delivery of PNA to cells and, to DNA targets within the cell nuclei. Nevertheless, recent developments in the PNA field give us optimism that in the near future, an anti-G-quadruplex PNA will be developed with good biological activity.

Acknowledgments

The study was partially sponsored by the Imaging Sciences Training Program supported in part by the Radiology and Imaging Sciences Department, Clinical Center and Intramural Research Program at the National Institute of Biomedical Imaging and Bioengineering, and supported by Intramural Research Programs of Clinical Center and National Institute of Diabetes and Digestive and Kidney Diseases, NIH.

References

1. Todd AK, Johnston M, Neidle S. Highly prevalent putative quadruplex sequence motifs in human DNA. Nucleic Acids Res. 2005; 33:2901–7. [PubMed: 15914666]
2. Huppert JL, Balasubramanian S. Prevalence of quadruplexes in the human genome. Nucleic Acids Res. 2005; 33:2908–16. [PubMed: 15914667]
3. Bryan TM, Baumann P. G-Quadruplexes: From Guanine Gels to Chemotherapeutics. Mol Biotechnol. 2011
4. Moyzis RK, Buckingham JM, Cram LS, et al. A highly conserved repetitive DNA sequence, (TTAGGG)n, present at the telomeres of human chromosomes. Proc Natl Acad Sci U S A. 1988; 85:6622–6. [PubMed: 3413114]
5. Eddy J, Vallur AC, Varma S, et al. G4 motifs correlate with promoter-proximal transcriptional pausing in human genes. Nucleic Acids Res. 2011; 39:4975–83. [PubMed: 21371997]
6. Eddy J, Maizels N. Conserved elements with potential to form polymorphic G-quadruplex structures in the first intron of human genes. Nucleic Acids Res. 2008; 36:1321–33. [PubMed: 18187510]
7. Eddy J, Maizels N. Gene function correlates with potential for G4 DNA formation in the human genome. Nucleic Acids Res. 2006; 34:3887–96. [PubMed: 16914419]
8. Brooks TA, Kendrick S, Hurley L. Making sense of G-quadruplex and i-motif functions in oncogene promoters. Febs J. 2010; 277:3459–69. [PubMed: 20670278]
9. Arola A, Vilar R. Stabilisation of G-quadruplex DNA by small molecules. Curr Top Med Chem. 2008; 8:1405–15. [PubMed: 18991726]
10. Haider SM, Neidle S, Parkinson GN. A structural analysis of G-quadruplex/ligand interactions. Biochimie. 2011
11. Huppert JL. Four-stranded nucleic acids: structure, function and targeting of G-quadruplexes. Chem Soc Rev. 2008; 37:1375–84. [PubMed: 18568163]
12. Stein CA, Cheng YC. Antisense oligonucleotides as therapeutic agents--is the bullet really magical? Science. 1993; 261:1004–12. [PubMed: 8351515]
13. Giovannangeli C, Helene C. Progress in developments of triplex-based strategies. Antisense Nucleic Acid Drug Dev. 1997; 7:413–21. [PubMed: 9303193]
14. Vaishnaw AK, Gollob J, Gamba-Vitalo C, et al. A status report on RNAi therapeutics. Silence. 2010; 1:14. [PubMed: 20615220]
15. Nielsen PE. Peptide nucleic acids (PNA) in chemical biology and drug discovery. Chem Biodivers. 2010; 7:786–804. [PubMed: 20397216]
16. Bukanov NO, Demidov VV, Nielsen PE, Frank-Kamenetskii MD. PD-loop: a complex of duplex DNA with an oligonucleotide. Proc Natl Acad Sci U S A. 1998; 95:5516–20. [PubMed: 9576914]

17. Datta B, Armitage BA. Hybridization of PNA to structured DNA targets: quadruplex invasion and the overhang effect. J Am Chem Soc. 2001; 123:9612–9. [PubMed: 11572682]
18. Lusvarghi S, Murphy CT, Roy S, et al. Loop and backbone modifications of peptide nucleic acid improve g-quadruplex binding selectivity. J Am Chem Soc. 2009; 131:18415–24. [PubMed: 19947597]
19. Roy S, Tanious FA, Wilson WD, Ly DH, Armitage BA. High-affinity homologous peptide nucleic acid probes for targeting a quadruplex-forming sequence from a MYC promoter element. Biochemistry. 2007; 46:10433–43. [PubMed: 17718513]
20. Amato J, Pagano B, Borbone N, et al. Targeting G-quadruplex structure in the human c-Kit promoter with short PNA sequences. Bioconjug Chem. 2011; 22:654–63. [PubMed: 21410246]
21. Onyshchenko MI, Gaynutdinov TI, Englund EA, Appella DH, Neumann RD, Panyutin IG. Stabilization of G-quadruplex in the BCL2 promoter region in double-stranded DNA by invading short PNAs. Nucleic Acids Res. 2009; 37:7570–80. [PubMed: 19820116]
22. Nielsen PE, Egholm M. An introduction to peptide nucleic acid. Curr Issues Mol Biol. 1999; 1:89–104. [PubMed: 11475704]
23. Nielsen PE, Egholm M, Berg RH, Buchardt O. Sequence specific inhibition of DNA restriction enzyme cleavage by PNA. Nucleic Acids Res. 1993; 21:197–200. [PubMed: 8382793]
24. Demidov VV, Potaman VN, Frank-Kamenetskii MD, et al. Stability of peptide nucleic acids in human serum and cellular extracts. Biochem Pharmacol. 1994; 48:1310–3. [PubMed: 7945427]
25. Pokorski JK, Witschi MA, Purnell BL, Appella DH. (S,S)-trans-cyclopentane-constrained peptide nucleic acids. a general backbone modification that improves binding affinity and sequence specificity. J Am Chem Soc. 2004; 126:15067–73. [PubMed: 15548003]
26. Dragulescu-Andrasi A, Rapireddy S, Frezza BM, Gayathri C, Gil RR, Ly DH. A simple gamma-backbone modification preorganizes peptide nucleic acid into a helical structure. J Am Chem Soc. 2006; 128:10258–67. [PubMed: 16881656]
27. Totsingan F, Tedeschi T, Sforza S, Corradini R, Marchelli R. Highly selective single nucleotide polymorphism recognition by a chiral (5S) PNA beacon. Chirality. 2009; 21:245–53. [PubMed: 18853465]
28. Debaene F, Da Silva JA, Pianowski Z, Duran FJ, Winssinger N. Expanding the scope of PNA-encoded libraries: divergent synthesis of libraries targeting cysteine, serine and metallo-proteases as well as tyrosine phosphatases. Tetrahedron. 2007; 63:6577–86.
29. Englund EA, Appella DH. Gamma-substituted peptide nucleic acids constructed from L-Iysine are a versatile scaffold for multifunctional display. Angew Chem Int Ed Eng 1. 2007; 46:1414–8.
30. Zhou P, Wang M, Du L, Fisher GW, Waggoner A, Ly DH. Novel binding and efficient cellular uptake of guanidine-based peptide nucleic acids (GPNA). J Am Chem Soc. 2003; 125:6878–9. [PubMed: 12783535]
31. Sahu B, Sacui I, Rapireddy S, et al. Synthesis and characterization of conformationally preorganized, (R)-diethylene glycol-containing gamma-peptide nucleic acids with superior hybridization properties and water solubility. J Org Chem. 2011; 76:5614–27. [PubMed: 21619025]
32. Cutrona G, Carpaneto EM, Ponzanelli A, et al. Inhibition of the translocated c-myc in Burkitt's lymphoma by a PNA complementary to the E mu enhancer. Cancer Research. 2003; 63:6144–8. [PubMed: 14559793]
33. Boffa LC, Scarfi S, Mariani MR, et al. Dihydrotestosterone as a selective cellular/nuclear localization vector for anti-gene peptide nucleic acid in prostatic carcinoma cells. Cancer Research. 2000; 60:2258–62. [PubMed: 10786693]
34. Rogers FA, Vasquez KM, Egholm M, Glazer PM. Site-directed recombination via bifunctional PNA-DNA conjugates. Proc Natl Acad Sci U S A. 2002; 99:16695–700. [PubMed: 12461167]
35. Wang G, Xu X, Pace B, et al. Peptide nucleic acid (PNA) binding-mediated induction of human gamma-globin gene expression. Nucleic Acids Res. 1999; 27:2806–13. [PubMed: 10373600]
36. McNeer NA, Chin JY, Schleifman EB, Fields RJ, Glazer PM, Saltzman WM. Nanoparticles deliver triplex-forming PNAs for site-specific genomic recombination in CD34+ human hematopoietic progenitors. Mol Ther. 2011; 19:172–80. [PubMed: 20859257]

37. Cutrona G, Carpaneto EM, Ulivi M, et al. Effects in live cells of a c-myc antigene PNA linked to a nuclear localization signal. Nature biotechnology. 2000; 18:300–3.
38. Cogoi S, Codognotto A, Rapozzi V, Meeuwenoord N, van der Marel G, Xodo LE. Transcription inhibition of oncogenic KRAS by a mutation-selective peptide nucleic acid conjugated to the PKKKRKV nuclear localization signal peptide. Biochemistry. 2005; 44:10510–9. [PubMed: 16060660]
39. Hu J, Corey DR. Inhibiting gene expression with peptide nucleic acid (PNA)--peptide conjugates that target chromosomal DNA. Biochemistry. 2007; 46:7581–9. [PubMed: 17536840]
40. Macadangdang B, Zhang N, Lund PE, et al. Inhibition of multidrug resistance by SV40 pseudovirion delivery of an antigene peptide nucleic acid (PNA) in cultured cells. PLoS One. 2011; 6:e17981. [PubMed: 21445346]
41. Kim KH, Nielsen PE, Glazer PM. Site-directed gene mutation at mixed sequence targets by psoralen-conjugated pseudo-complementary peptide nucleic acids. Nucleic Acids Res. 2007; 35:7604–13. [PubMed: 17977869]
42. Birkedal H, Nielsen PE. Targeted gene correction using psoralen, chlorambucil and camptothecin conjugates of triplex forming peptide nucleic acid (PNA). Artif DNA PNA XNA. 2011; 2:23–32. [PubMed: 21686249]
43. Demidov VV, Frank-Kamenetskii MD. Two sides of the coin: affinity and specificity of nucleic acid interactions. Trends Biochem Sci. 2004; 29:62–71. [PubMed: 15102432]
44. Mirkin SM. Discovery of alternative DNA structures: a heroic decade (1979–1989). Frontiers in bioscience: a journal and virtual library. 2008; 13:1064–71. [PubMed: 17981612]
45. Venkadesh S, Mandal PK, Gautham N. The structure of a full turn of an A-DNA duplex d(CGCGGGTACCCGCG). Biochem Biophys Res Commun. 2011; 407:307–12. [PubMed: 21397589]
46. Bae S, Kim D, Kim KK, Kim YG, Hohng S. Intrinsic Z-DNA is stabilized by the conformational selection mechanism of Z-DNA-binding proteins. J Am Chem Soc. 2011; 133:668–71. [PubMed: 21171590]
47. Xue L, Xi H, Kumar S, et al. Probing the recognition surface of a DNA triplex: binding studies with intercalator-neomycin conjugates. Biochemistry. 2010; 49:5540–52. [PubMed: 20499878]
48. Dai J, Hatzakis E, Hurley LH, Yang D. I-motif structures formed in the human c-MYC promoter are highly dynamic--insights into sequence redundancy and I-motif stability. PLoS One. 2010; 5:e11647. [PubMed: 20657837]
49. Panyutin IG, Kovalsky OI, Budowsky EI, Dickerson RE, Rikhirev ME, Lipanov AA. G-DNA: a twice-folded DNA structure adopted by single-stranded oligo(dG) and its implications for telomeres. Proc Natl Acad Sci U S A. 1990; 87:867–70. [PubMed: 2300578]
50. Mathad RI, Yang D. G-quadruplex structures and G-quadruplex-interactive compounds. Methods Mol Biol. 2011; 735:77–96. [PubMed: 21461813]
51. Chou SH, Chin KH, Wang AH. Unusual DNA duplex and hairpin motifs. Nucleic Acids Res. 2003; 31:2461–74. [PubMed: 12736295]
52. Frank-Kamenetskii MD, Mirkin SM. Triplex DNA structures. Annu Rev Biochem. 1995; 64:65–95. [PubMed: 7574496]
53. Bissler JJ. Triplex DNA and human disease. Front Biosci. 2007; 12:4536–46. [PubMed: 17485395]
54. Shalaby T, Hiyama E, Grotzer MA. Telomere maintenance as therapeutic target in embryonal tumours. Anticancer Agents Med Chem. 2010; 10:196–212. [PubMed: 20017721]
55. Wu Y, Brosh RM Jr. G-quadruplex nucleic acids and human disease. Febs J. 2010; 277:3470–88. [PubMed: 20670277]
56. Nielsen PE, Egholm M, Berg RH, Buchardt O. Sequence-selective recognition of DNA by strand displacement with a thymine-substituted polyamide. Science. 1991; 254:1497–500. [PubMed: 1962210]
57. Egholm M, Christensen L, Dueholm KL, Buchardt O, Coull J, Nielsen PE. Efficient pH-independent sequence-specific DNA binding by pseudoisocytosine-containing bis-PNA. Nucleic Acids Res. 1995; 23:217–22. [PubMed: 7862524]
58. Demidov VV, Frank-Kamenetskii MD. PNA openers and their applications. Methods in molecular biology. 2002; 208:119–30. [PubMed: 12229284]

59. Panyutin IG, Panyutin IV, Demidov VV. Targeting linear duplex DNA with mixed-base peptide nucleic acid oligomers facilitated by bisPNA openers. Analytical biochemistry. 2007; 362:145–7. [PubMed: 17184722]
60. Datta B, Bier ME, Roy S, Armitage BA. Quadruplex formation by a guanine-rich PNA oligomer. J Am Chem Soc. 2005; 127:4199–207. [PubMed: 15783201]
61. Datta B, Schmitt C, Armitage BA. Formation of a PNA2-DNA2 hybrid quadruplex. J Am Chem Soc. 2003; 125:4111–8. [PubMed: 12670232]
62. Englund EA, Xu Q, Witschi MA, Appella DH. PNA-DNA duplexes, triplexes, and quadruplexes are stabilized with trans-cyclopentane units. J Am Chem Soc. 2006; 128:16456–7. [PubMed: 17177367]
63. Esposito V, Galeone A, Mayol L, Messere A, Piccialli G, Randazzo A. PNA-DNA chimeras forming quadruplex structures. Nucleosides Nucleotides Nucleic Acids. 2003; 22:1681–4. [PubMed: 14565494]
64. Paul A, Sengupta P, Krishnan Y, Ladame S. Combining G-quadruplex targeting motifs on a single peptide nucleic acid scaffold: a hybrid (3+1) PNA-DNA bimolecular quadruplex. Chemistry. 2008; 14:8682–9. [PubMed: 18668497]
65. Amato J, Gabelica V, Borbone N, et al. A short C-rich PNA fragment capable to form novel G-quadruplex-PNA complexes. Nucleic Acids Symp Ser (Oxf). 2008:167–8.
66. Amato J, Oliviero G, De Pauw E, Gabelica V. Hybridization of short complementary PNAs to G-quadruplex forming oligonucleotides: An electrospray mass spectrometry study. Biopolymers. 2009; 91:244–55. [PubMed: 19065573]
67. Onyshchenko MI, Gaynutdinov TI, Englund EA, Appella DH, Neumann RD, Panyutin IG. Quadruplex formation is necessary for stable PNA invasion into duplex DNA of BCL2 promoter region. Nucleic Acids Res. 2011
68. Zhang X, Ishihara T, Corey DR. Strand invasion by mixed base PNAs and a PNA-peptide chimera. Nucleic Acids Res. 2000; 28:3332–8. [PubMed: 10954602]
69. Amiard S, Doudeau M, Pinte S, et al. A topological mechanism for TRF2-enhanced strand invasion. Nature structural & molecular biology. 2007; 14:147–54.
70. Qin Y, Rezler EM, Gokhale V, Sun D, Hurley LH. Characterization of the G-quadruplexes in the duplex nuclease hypersensitive element of the PDGF-A promoter and modulation of PDGF-A promoter activity by TMPyP4. Nucleic Acids Res. 2007; 35:7698–713. [PubMed: 17984069]
71. Qin Y, Hurley LH. Structures, folding patterns, and functions of intramolecular DNA G-quadruplexes found in eukaryotic promoter regions. Biochimie. 2008; 90:1149–71. [PubMed: 18355457]
72. Sun D, Guo K, Rusche JJ, Hurley LH. Facilitation of a structural transition in the polypurine/polypyrimidine tract within the proximal promoter region of the human VEGF gene by the presence of potassium and G-quadruplex-interactive agents. Nucleic Acids Res. 2005; 33:6070–80. [PubMed: 16239639]
73. Guo K, Gokhale V, Hurley LH, Sun D. Intramolecularly folded G-quadruplex and i-motif structures in the proximal promoter of the vascular endothelial growth factor gene. Nucleic Acids Res. 2008; 36:4598–608. [PubMed: 18614607]
74. Siddiqui-Jain A, Grand CL, Bearss DJ, Hurley LH. Direct evidence for a G-quadruplex in a promoter region and its targeting with a small molecule to repress c-MYC transcription. Proc Natl Acad Sci U S A. 2002; 99:11593–8. [PubMed: 12195017]
75. Cogoi S, Paramasivam M, Spolaore B, Xodo LE. Structural polymorphism within a regulatory element of the human KRAS promoter: formation of G4-DNA recognized by nuclear proteins. Nucleic Acids Res. 2008; 36:3765–80. [PubMed: 18490377]
76. Rankin S, Reszka AP, Huppert J, et al. Putative DNA quadruplex formation within the human c-kit oncogene. J Am Chem Soc. 2005; 127:10584–9. [PubMed: 16045346]
77. Hsu ST, Varnai P, Bugaut A, Reszka AP, Neidle S, Balasubramanian S. A G-rich sequence within the c-kit oncogene promoter forms a parallel G-quadruplex having asymmetric G-tetrad dynamics. J Am Chem Soc. 2009; 131:13399–409. [PubMed: 19705869]

78. Dexheimer TS, Sun D, Hurley LH. Deconvoluting the structural and drug-recognition complexity of the G-quadruplex-formingregion upstream of the bcl-2 P1 promoter. J Am Chem Soc. 2006; 128:5404–15. [PubMed: 16620112]

79. Dai J, Chen D, Jones RA, Hurley LH, Yang D. NMR solution structure of the major G-quadruplex structure formed in the human BCL2 promoter region. Nucleic Acids Res. 2006; 34:5133–44. [PubMed: 16998187]

80. Palumbo SL, Ebbinghaus SW, Hurley LH. Formation of a unique end-to-end stacked pair of G-quadruplexes in the hTERT core promoter with implications for inhibition of telomerase by G-quadruplex-interactive ligands. J Am Chem Soc. 2009; 131:10878–91. [PubMed: 19601575]

81. Xu Y, Sugiyama H. Formation of the G-quadruplex and i-motif structures in retinoblastoma susceptibility genes (Rb). Nucleic Acids Res. 2006; 34:949–54. [PubMed: 16464825]

82. Qin Y, Fortin JS, Tye D, Gleason-Guzman M, Brooks TA, Hurley LH. Molecular cloning of the human platelet-derived growth factor receptor beta (PDGFR-beta) promoter and drug targeting of the G-quadruplex-forming region to repress PDGFR-beta expression. Biochemistry. 2010; 49:4208–19. [PubMed: 20377208]

83. Sun D, Hurley LH. The importance of negative superhelicity in inducing the formation of G-quadruplex and i-motif structures in the c-Myc promoter: implications for drug targeting and control of gene expression. J Med Chem. 2009; 52:2863–74. [PubMed: 19385599]

84. Gonzalez V, Hurley LH. The C-terminus of nucleolin promotes the formation of the c-MYC G-quadruplex and inhibits c-MYC promoter activity. Biochemistry. 2010; 49:9706–14. [PubMed: 20932061]

85. Duquette ML, Handa P, Vincent JA, Taylor AF, Maizels N. Intracellular transcription of G-rich DNAs induces formation of G-loops, novel structures containing G4 DNA. Genes & development. 2004; 18:1618–29. [PubMed: 15231739]

86. Basundra R, Kumar A, Amrane S, Verma A, Phan AT, Chowdhury S. A novel G-quadruplex motif modulates promoter activity of human thymidine kinase 1. Febs Journal. 2010; 277:4254–64. [PubMed: 20849417]

87. Sanders CM. Human Pif1 helicase is a G-quadruplex DNA-binding protein with G-quadruplex DNA-unwinding activity. Biochemical Journal. 2010; 430:119–28. [PubMed: 20524933]

88. Cogoi S, Paramasivam M, Membrino A, Yokoyama KK, Xodo LE. The KRAS Promoter Responds to Myc-associated Zinc Finger and Poly(ADP-ribose) Polymerase 1 Proteins, Which Recognize a Critical Quadruplex-forming GA-element. Journal of Biological Chemistry. 2010; 285:22003–16. [PubMed: 20457603]

89. Fernando H, Sewitz S, Darot J, Tavare S, Huppert JL, Balasubramanian S. Genome-wide analysis of a G-quadruplex-specific single-chain antibody that regulates gene expression. Nucleic Acids Res. 2009; 37:6716–22. [PubMed: 19745055]

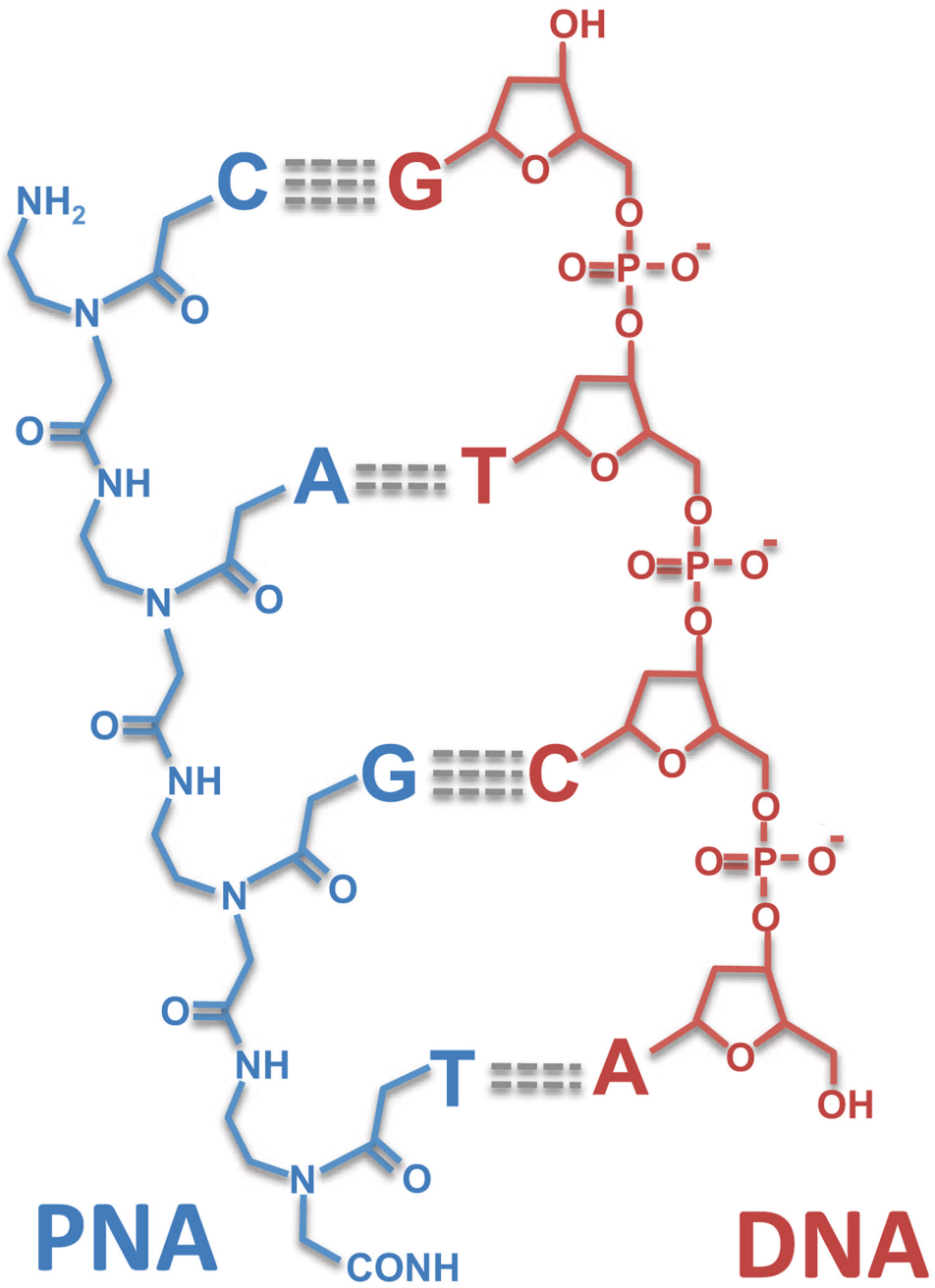

Figure 1.
PNA (blue) and DNA (red) backbones. Shown PNA-DNA hybrid.

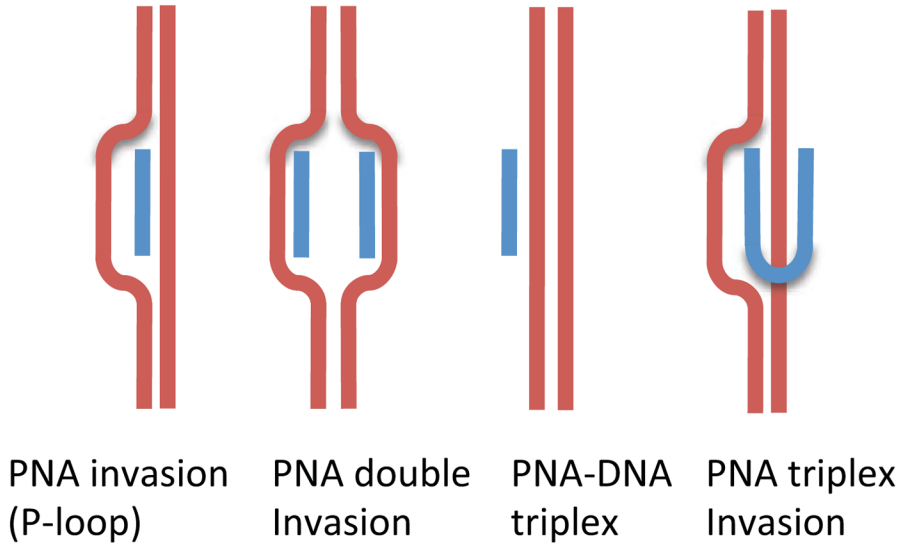

Figure 2.
Various modes of PNA (blue) binding to duplex DNA (red).

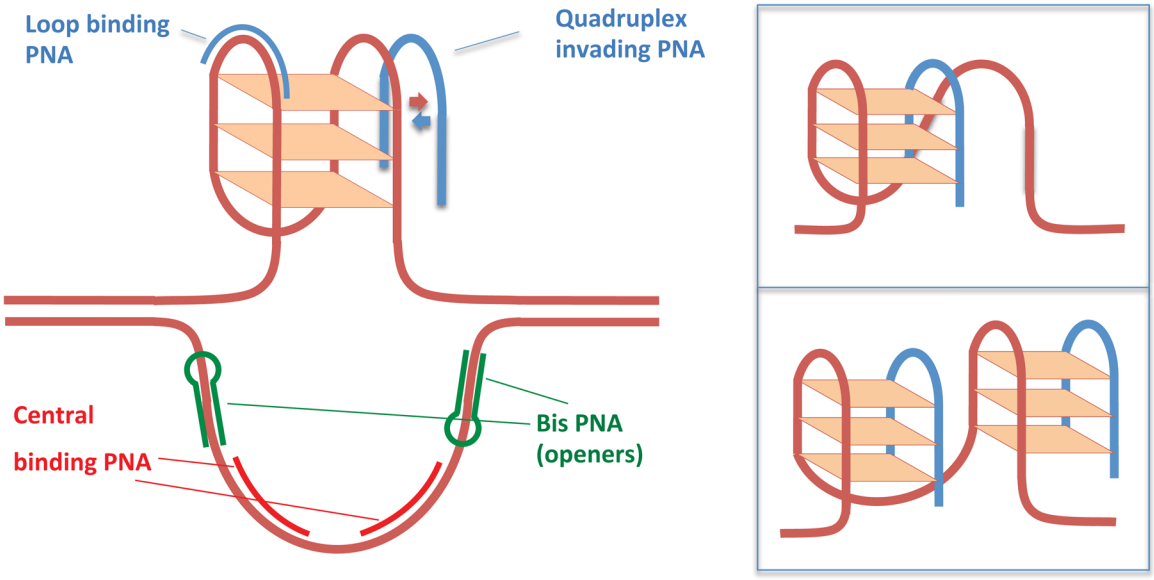

Figure 3.
Proposed modes of PNA binding to DNA G-quadruplex formed in duplex DNA. Quadruplex binding PNA (shown in dashed) replaces one or more DNA strands containing quadruplex-core-forming runs of guanines.

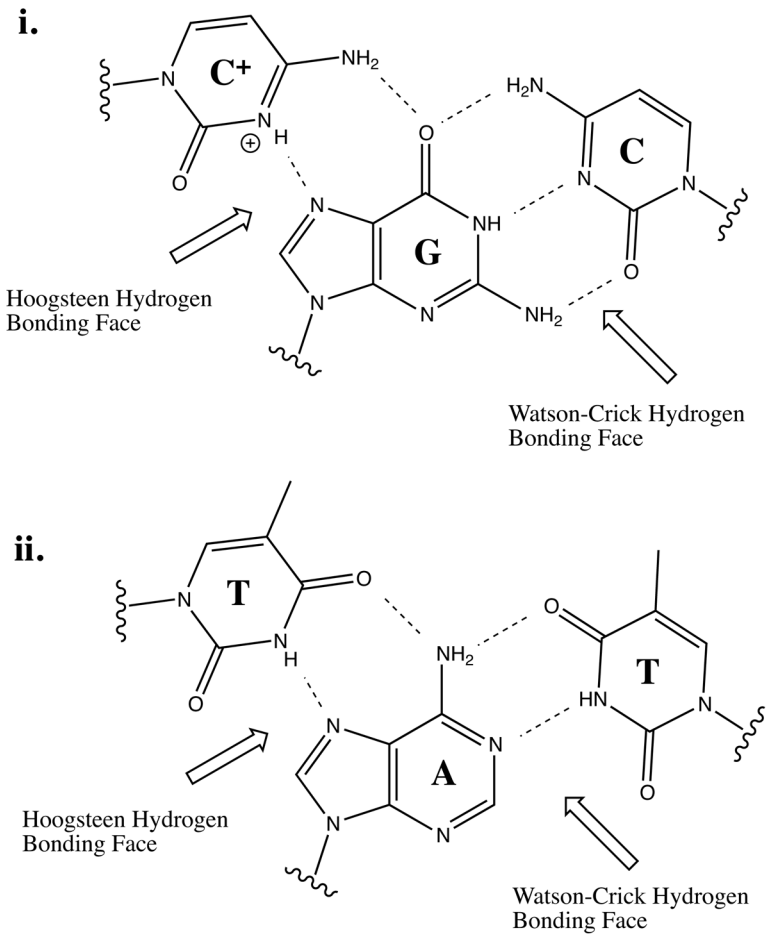

Figure 4.

(i). The hydrogen bonding formation of triplex form nucleic acids. The guanine:cytosine form a pair on the Watson-Crick face of the guanine forming a duplex as third oligomer containing a protonated cytosine binds the Hoogsteen face of guanine. The third strand would be stationed in the major groove of normal nucleic acid duplex. (ii). The same type base-pairing with complementary thymine residues across both Hoogsteen and Watson-Crick faces of the adenine.

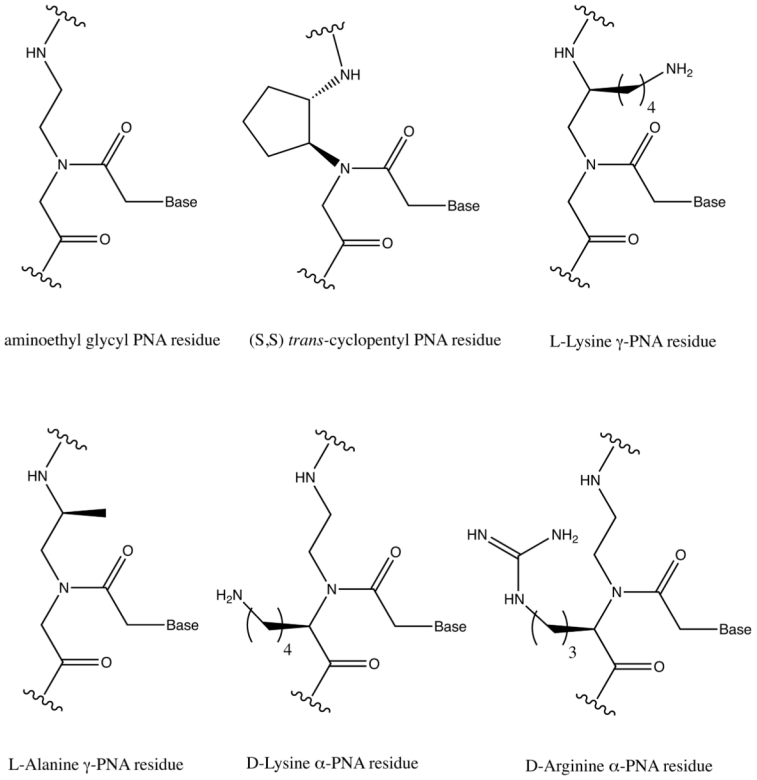

Figure 5.

The composition of aminoethyl glycine PNA (aegPNA) and some of the common derivatives based on maintaining the basic form of the PNA backbone while augmenting it with ring structures or side chains.

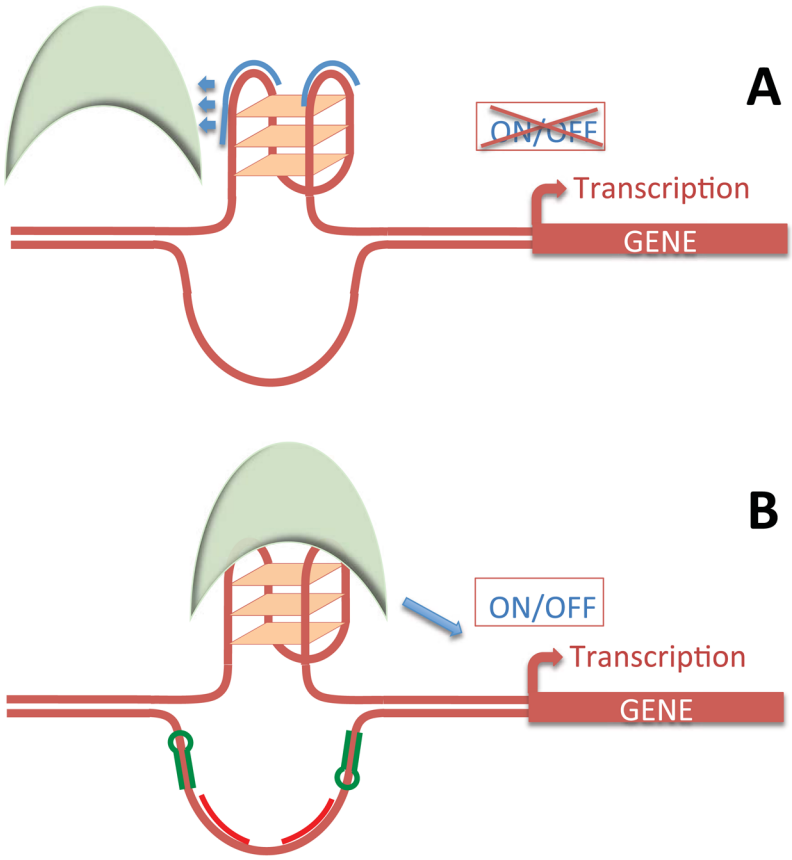

Figure 6.
Possible effects of PNA binding to a quadruplex-forming sequence on G-quadruplex recognition by a protein factor and gene transcription. A. PNA bind G-quadruplex-forming sequence interfering with a protein factor (green) recognition of the quadruplex; gene regulation by the protein factor is disrupted. B. PNA bind cytosine-rich strand complementary to G-quadruplex-forming sequence, thus, helping quadruplex formation. A protein factor recognizes the quadruplex, binds to it and accomplishes its transcription regulation functions.