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REVIEW ARTICLE

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Versatility of peptide nucleic acids (PNAs): role in chemical biology, drug discovery, and origins of life*

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This review briefly discussed nomenclature, synthesis, chemistry, and biophysical properties of a plethora of PNA derivatives reported since the discovery of aegPNA. Different synthetic methods and structural analogs of PNA synthesized till date were also discussed. An insight was gained into various chemical, physical, and biological properties of PNA which make it preferable over all other classes of modified nucleic acid analogs. Thereafter, various approaches with special attention to the practical constraints, characteristics, and inherent drawbacks leading to the delay in the development of PNA as gene therapeutic drug were outlined. An explicit account of the successful application of PNA in different areas of research such as antisense and antigene strategies, diagnostics, molecular probes, and so forth was described along with the current status of PNA as gene therapeutic drug. Further, the plausibility of the existence of PNA and its role in primordial chemistry, that is, origin of life was explored in an endeavor to comprehend the mystery and open up its deepest secrets ever engaging and challenging the human intellect. We finally concluded it with a discussion on the future prospects of PNA technology in the field of therapeutics, diagnostics, and origin of life.

KEYWORDS
chemical biology, food analysis, gene therapy, nanotechnology, origin of life, peptide nucleic acid

1 | INTRODUCTION

1.1 | History of synthetic oligonucleotides

Advances in understanding nature's simple and elegant language and molecular mechanism with which it communicates the cryptic genetic information have intrigued and fascinated the scientists and researchers all over the world. It has also spurred research efforts dedicated to the development of various drugs to ameliorate or cure diseases at the genetic level. The concept underlying the transfer of genetic information from DNA to protein[1] provides the possibility of rational design of short nucleic acid fragments (oligonucleotides) in future as therapeutic agents and biomolecular tools.

Scientists have been working on many strategies since the discovery of antisense and antigene technology and have formulated various methods to selectively turn off specific genes of diseased cell. Pertinent references date back to 1960s when Belivoka *et al.* synthesized nucleoside and dinucleoside phosphate derivatives which selectively bind to tRNA.[2] In 1970s, Paterson and coworkers demonstrated a simple method to inhibit the translation of RNA and also for precisely mapping structural gene sequences within DNA.[3] Stephenson and Zamecnik also reported the potential of a tridecamer oligodeoxynucleotide to inhibit translation of RNA in Rous sarcoma virus.[4] Basically, an oligonucleotide analog complimentary to a RNA or DNA sequence was used as a ligand to bind dsDNA with high affinity and selectivity.

With the advent of technological advancement making available the automated synthesizers and development of newer efficient synthetic chemistry, scientists were able to synthesize various new chemically modified oligonucleotides with ease. Despite the accelerated research pursued

*Dedicated to PNA inventor Prof. Peter E. Nielsen

by various groups modifying DNA molecule either at phosphate unit or at sugar unit, no research group tried to replace both sugar and phosphate unit at the same time and much was still to be done to make them suitable for use as therapeutic agent (Figure 1).[5–7] Interestingly, in 1987, Westheimer inquisitive to know why nature chose phosphate esters and anhydrides which are ubiquitous in the biochemistry and living world suggested ethylenediamine monoacetic acid along with other possible replacements for the ribose phosphate.[8] It was difficult to predict all properties and applications resulting from a neutral backbone. However, it was fascinating scientific endeavor to develop a mimic of nucleic acids with a neutral backbone. It was apparent that the resulting molecule would possess potential applications in medicine and chemical biology.

1.2 | Twist in the tale, replacing a backbone

It was well established that purine and pyrimidine oligonucleotides bind specifically through T·A-T and C^+·G-C or G·G-C and A·A-T triplets to homopurine regions of dsDNA by triple helix formation.[9–13] Nielsen and coworkers designed a new triple helix-forming oligonucleotide to bind dsDNA via Hoogsteen base pairing in the major groove by computer aided model by detaching the backbone of one strand in a B-DNA duplex and replacing it with amino acid units. They considered various criteria such as rigidity, water solubility, and chemical accessibility. In 1991, they reported an invaluable discovery of an unprecedented synthetic oligomer in which sugar–phosphate backbone was completely replaced by 2-aminoethyl glycine (aeg).[14] The nucleobases were attached via carboxymethylene linker to the nitrogen of pseudopeptide backbone. These modifications did not alter configuration and the distance between two nucleobases. Thus, the resulting PNA molecule was homomorphous to DNA with high degree of constrained conformational flexibility due to two amido groups per unit. Surprisingly, the strand displacement of A-strand with displacement of the opposite T-strand took place on binding the homothymine PNA decamer targeted to a $(dA/dT)_{10}$ target in a 248-base pair dsDNA.[14]

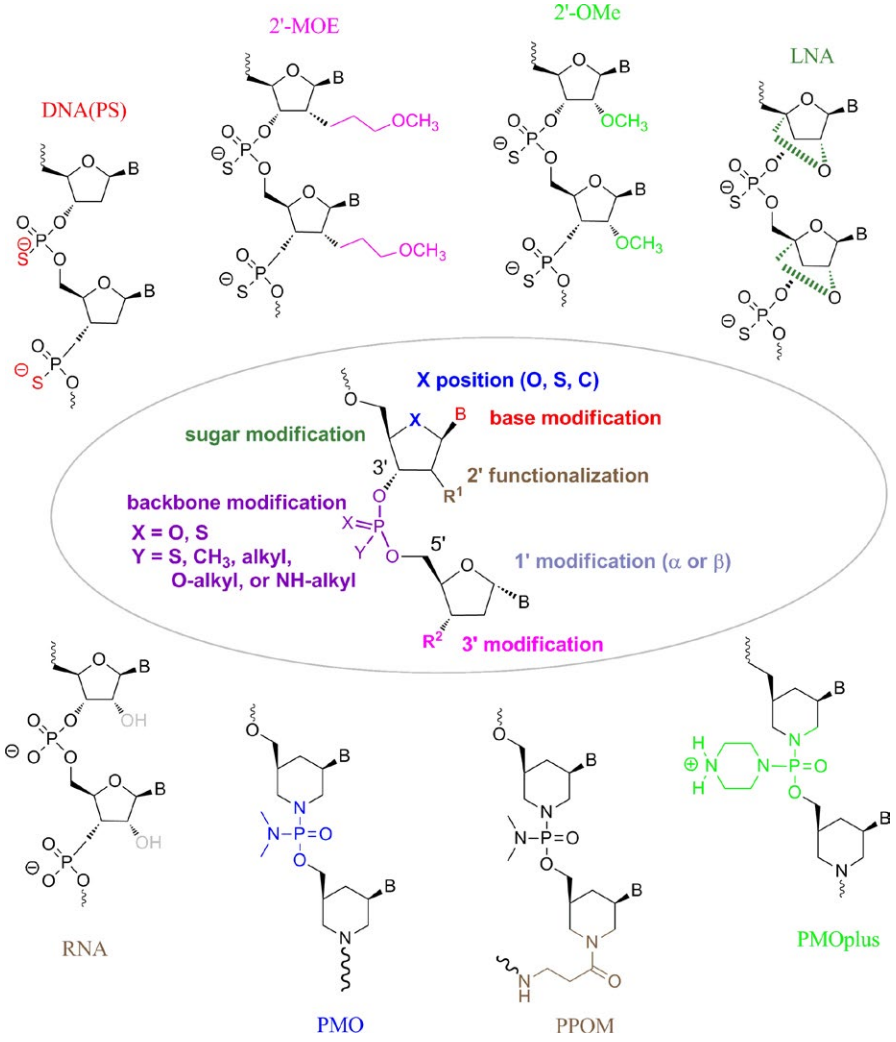


FIGURE 1 Various modifications in DNA

1.3 | Applications and versatility of fertile PNA

PNA is a versatile synthetic pseudopeptide with many applications. It undoubtedly has surpassed natural oligonucleotides with unrivaled performance to become foremost in the development of newer approaches in a wide area of research. It was initially proposed to bind to the major groove of dsDNA by Hoogsteen-like hydrogen bonding forming a triplex. However, first experiment with homothymine PNA revealed its ability to bind sequence specifically to dsDNA (Figure 2). Further, the formation of triplex with homoadenine target in dsDNA with the displacement of homothymine target in DNA was reported.[14]

1.4 | Review coverage

We searched the online database ('Scifinder Scholar': scifinder.cas.org) for the existing literature on PNA. One common observation on the reviews already present in the literature is that the reviews published in the last decade have focused on specialized aspects of PNA either their applications or chemistry. Thus, no review has been published so far which is all in one. Hence, we reviewed the importance of PNA in chemistry, molecular biology, drug discovery, diagnostics, and origin of life.

We began with the history of synthetic oligonucleotides and detailed discussion mentioning how replacing a backbone resulted in surprising results of versatile PNA. We have also included the nomenclature of plethora of PNA analogs and derivatives that were synthesized over a period of time to improve properties of PNAs for various applications. Then, we discussed various synthetic protocols used for the synthesis of PNA monomers, modified monomers, and its oligomerization in both solution and solid phase. We then highlighted and compared unique features of PNA in detail which makes them superior than most of the contemporary DNA mimics of its time or earlier. The biological properties of PNA which makes it suitable for antisense and antigene applications have been discussed. We have also shed light on various approaches for cellular delivery of PNA including microjection, electroporation, use of cell penetration peptides, use of cationic lipids, and interaction of DNA in detail which will provide overall view of development in cellular delivery of PNA. Further, a broad overview on all the applications of PNA has been given. Judging from the expanding literature in this field, we have concluded this review by critically analyzing the successes and failures in PNA research.

2 | NOMENCLATURE

Nielsen and his group coined the term 'PNA' to emphasize relatively straightforward synthesis of monomers and PNA oligomers using SPPS and due to its obvious relationship to nucleic acids.[15] This is not strictly a chemically correct name. Rather, PNA is a misnomer as it is neither a peptide nor a nucleic acid, but a combination of both. Generally, PNAs are written like peptides, starting from N- to C-terminal, with the N-terminus written first at left position and the C-terminus at the right. For example, the sequence of first synthetic acridine functionalized decamer of thymine with lysinyl amide attached to the C-terminus is written as Acr-T_{10}-Lys-NH_2.[14]

When the N-terminal of PNA hybridizes with 5′-end of the corresponding oligonucleotide strand, it is called parallel orientation and a duplex with 3′-end of oligonucleotide adjacent to the N-terminus of PNA is known as antiparallel. The atoms and the backbone torsion angles are named similar to isomorphic nucleic acids (Figure 3).

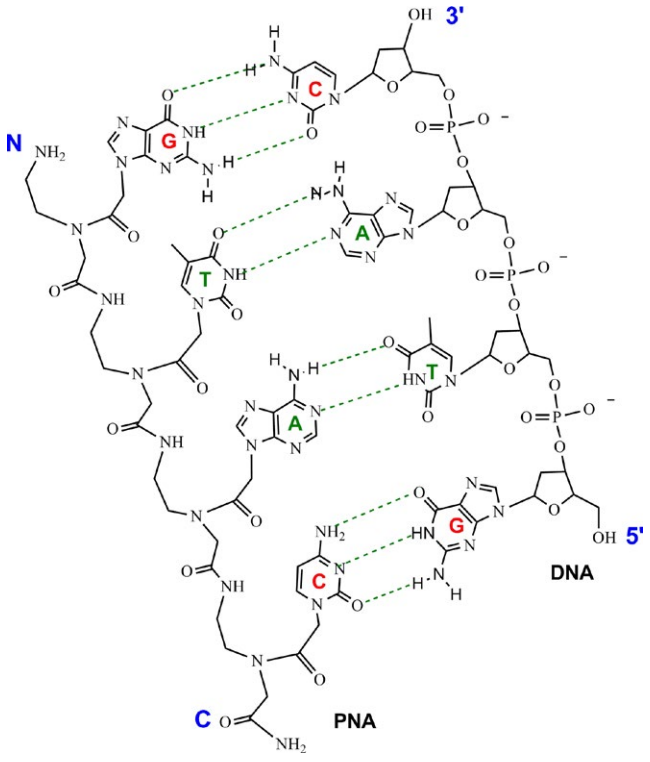


FIGURE 2 PNA–DNA duplex (antiparallel)

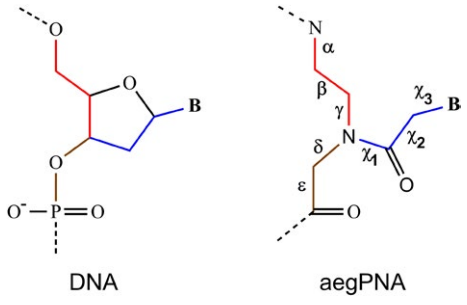


FIGURE 3 Structures of PNA and DNA monomers

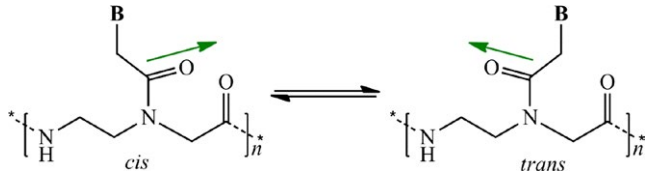


FIGURE 4 Two rotameric structures of ssPNA

Two rotameric structures are found to coexist about the tertiary amide bond in monomers and ssPNA; if the carbonyl group of the backbone-base linker is found to point toward the C-terminus, it is named *cis*-PNA, and the other rotameric form is known as *trans*-PNA (Figure 4).[16]

2.1 | Naming modified PNAs (A to Z of PNA)

The chemical structure of PNA backbone, due to its inherent constrained flexibility, is responsible for its hybridization and excellent nucleic acid mimicking properties. However, poor membrane permeability and tendency to self-aggregation hamper the usefulness of this molecule in various applications. These problems stimulated chemists to develop modified PNAs with superior properties to aegPNA. Their binding affinity with DNA and RNA can be enhanced by suitable preorganization of PNA backbone by rigidification and cyclization. Addition of substituents and introduction of chirality to the backbone influences the selectivity in binding. Subsequently, the backbone, nucleobases, and the linker connecting a nucleobase to the backbone have been modified. Hence, a plethora of PNA analogs and derivatives have been designed and synthesized to make PNAs with improved properties for various applications and to study new applications. The straightforward synthetic protocol used in the modification of PNAs makes them easily accessible. The modified chiral PNAs bearing substituents on backbones can be divided into α-PNA,[17] β-PNA,[18] and γ-PNA[19] based on the position of a substituent in the PNA backbone (Figure 5).

Many modifications in the backbone and nucleobases are also done, and new conjugates are denoted differently as shown in Tables 1–3.[14,20–64]

3 | SYNTHESES

Although PNA monomers and customized oligomers are commercially available, PNA is synthesized easily by well-established automated solid-phase synthetic procedures. Versatile synthesis of peptide nucleic acids allows relatively large-scale production in high yield and purity by oligomerization of different monomers on solid support by standard SPPS protocols.[65,66] Fewer syntheses with solution phase are also reported.[67–70] As the backbone of PNA is based on poly amides, PNA can be easily linked to peptides to add functionality. PNA oligomers can also be labeled with dyes or other chemicals, and linkers can be added as a spacer. Postsynthesis PNA oligomers can be cleaved from the solid support by employing traditional peptide chemistry. Crude PNAs can be purified by reverse-phase high-performance liquid chromatography and characterized by mass spectrometry.

3.1 | PNA monomer syntheses

PNA monomers are synthesized by convergent syntheses.[71] Generally, a suitably protected base and N-protected pseudopeptide backbone (2-aminoethyl glycine) is synthesized. Then, the backbone is coupled with PNA nucleobase acetic acid to get the desired PNA monomers (Figure 6).

However, the protecting groups attached to the backbone and the nucleobase should be orthogonal; that is, nucleobase protecting group must be stable to the conditions used to remove the NH-protecting group attached to backbone. Different protection schemes, viz. Boc, Fmoc, and Mmt, are used from the combination of several protecting groups. Originally, homothymine PNA decamer was synthesized on a Merrifield MBHA resin using the Boc/Cbz strategy.[65,71,72] Later, better and easy Fmoc chemistry[73,74] was developed and the Mmt monomers[75] have also been used recently. Boc/Cbz and Fmoc/Bhoc PNA monomers are commercially available and are used for routine PNA synthesis with the possibility of using commercial peptide synthesizers.

The self-activated cyclic Bts PNA monomers were also developed using benzothiazole-2-sulfonyl as an amine-protecting group.[76] The reported synthesis employs reaction of ethyl *N*-(2-aminoethyl)glycinate with benzothiazole-2-sulfonyl chloride. Bhoc, due to an ease of deprotection and stability toward nucleophiles, was selected for the protection of exocyclic amines of the nucleobases. The Bts-protected backbone and Bhoc-protected base acetic acid were coupled using DCC/HOBt, followed by hydrolysis

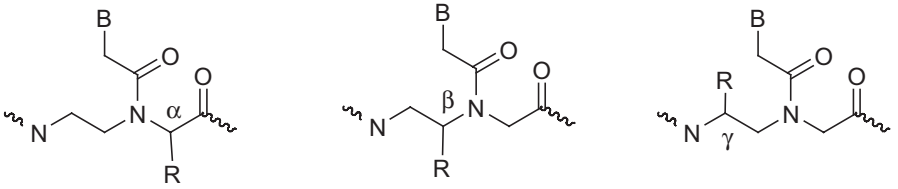


FIGURE 5 Structures of α-, β-, and γ-PNA monomers

TABLE 1 Backbone modifications in PNAs

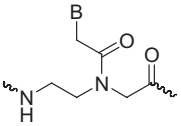
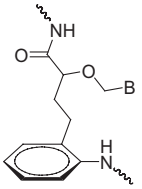
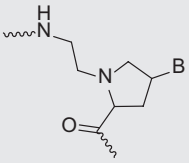
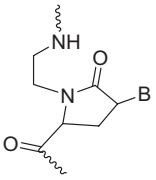
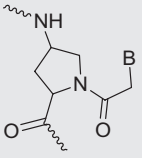
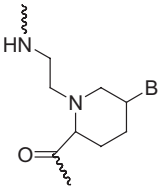
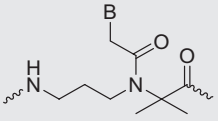
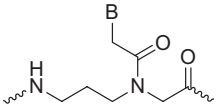
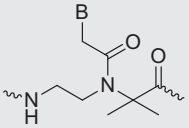
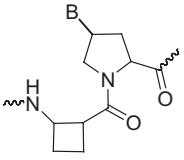
Sl. no	Abbreviation	Name (expanded)	Structure/detail	References
1.	*aeg*-PNA	Aminoethtyl glycine PNA		[14]
Backbone modifications				
2.	APNA	Aromatic peptide nucleic acids		[20]
3.	*aep* PNA	Amino ethyl prolyl PNA		[21]
4.	*aepone*-PNA	Amino ethyl pyrrolidinone PNA		[22]
5.	*ap*-PNA and amp-PNA	Amino prolyl PNA		[23,24]
6.	*aepip*-PNA	Amino ethyl pipecolyl PNA		[25]
7.	apdmg-PNA	Amino propyl dimethyl glycyl PNA		[26]
8.	Apg-PNA	Amino propyl glycinyl PNA		[26]
9.	aedmg PNA	Amino ethyl dimethyl glycyl PNA		[26]
10.	acbc-PNA	2-Amino cyclo butane carboxylic PNA		[27]

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TABLE 1 (continued)

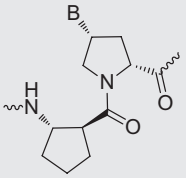
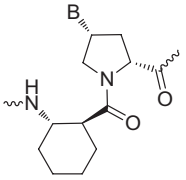
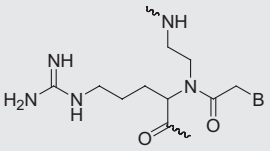
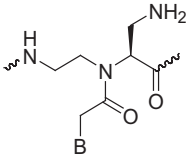
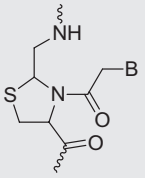
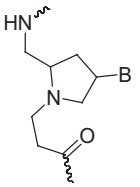
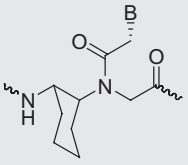
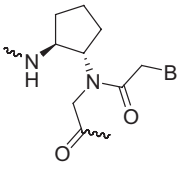
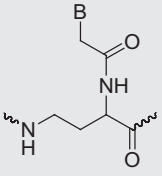
Sl. no	Abbreviation	Name (expanded)	Structure/detail	References
11.	acpc-PNA	Amino cyclo pentane carboxylic PNA		[28]
12.	achc-PNA	2-Amino cyclo hexane carboxylic PNA		[27]
13.	arg-PNA	Arginine-derived PNAs		[29]
14.	am-PNA	Amino methylene PNA		[30]
15.	Amt-PNA	Amino methyl thiazolidine PNA		[31]
16.	bepPNA	Backbone-extended pyrrolidine PNA		[32]
17.	ch-PNA	Cyclohexyl PNA		[33]
18.	cp-PNA, cyp-PNA	Cyclopentyl PNA, trans-cyclopentylPNA		[34]
19.	Dab PNA	Diaminobutyryl-PNA		[35]

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TABLE 1 (continued)

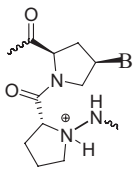
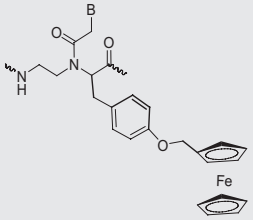
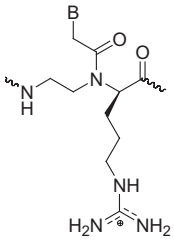
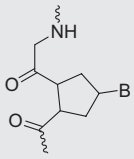
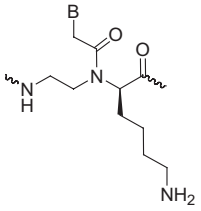
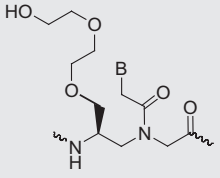
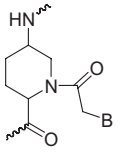
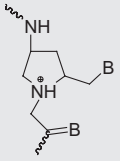
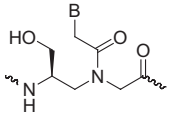
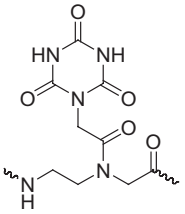
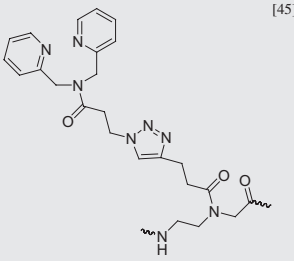
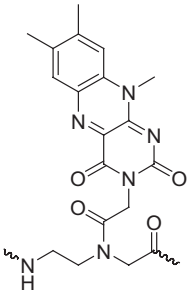
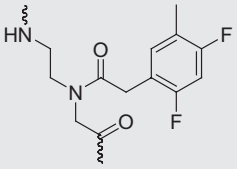
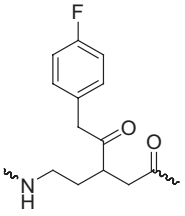
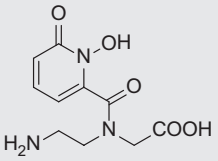
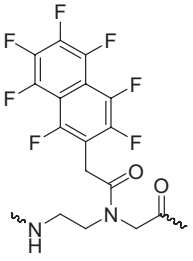
Sl. no	Abbreviation	Name (expanded)	Structure/detail	References
20.	D-apc PNA	1-Amino pyrrolidine carboxylic acid PNA		[36]
21.	FcPNA	Ferrocene-labeled PNA		[37]
22.	GPNA	Guanidine-based peptide nucleic acids		[38]
23.	Gly-pro-PNA	Prolyl glycyl PNA		[39]
24.	^{D}Kα-PNA	D-Lysine α-PNA L-Lysine γ-PNA		[40]
25.	$^{R\text{-}MP}$γPNA	(R)-MiniPEG γ-PNA		[41]
26.	*pip*-PNA	Pipecolyl PNA		[42]
27.	*Pyr* PNA	Pyrrolidine PNA		[43]
28.	^{L}Sγ-PNA	L-Serine γ-PNA		[19]

TABLE 2 Nucleobase modifications in PNAs

Sl. no	Abbreviation	Name (expanded)	Structure/detail	References
1.	CyaPNA	Cyanuryl PNA		[44]
2.	DpamPNA	Di-(2-picolyl)amide PNA		[45]
3.	FlavPNA	Flavin-containing PNA		[46]
4.	FPNA	Fluorine-modified PNA		[47]
5.	4-FPh PNA	4-Fluorophenyl PNA		[48]
6.	HOPO-PNAm	1,2-Hydroxypyridinone PNA monomer		[49]
7.	β-HFN PNA	β-Hepta fluoro naphthalene PNA		[48]

(continues)

TABLE 2 (continued)

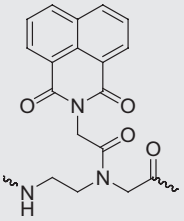
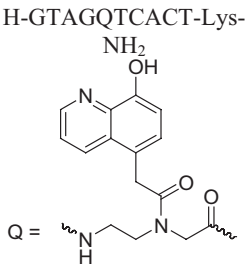
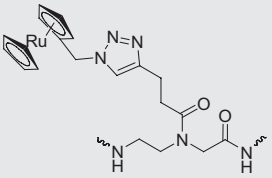
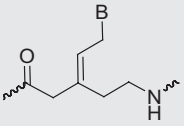
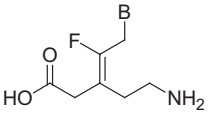
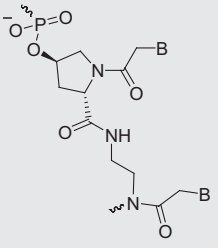
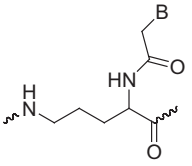
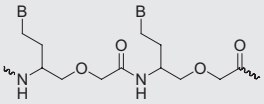
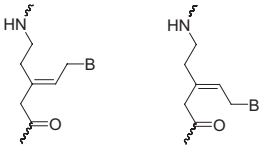
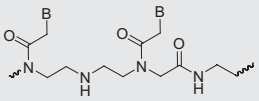
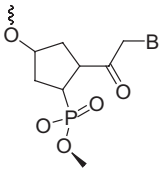
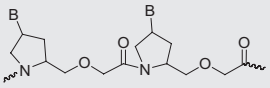
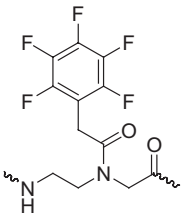
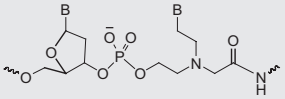
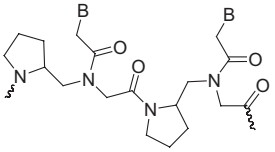
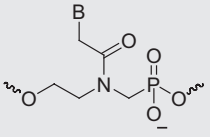
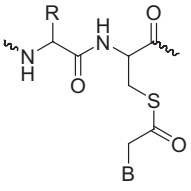
Sl. no	Abbreviation	Name (expanded)	Structure/detail	References
8.	NPNA	Naphthalimide-containing PNA		[50]
9.	Q-PNA	Quencher labeled PNA (e.g., 8-hydroxyquinoline Q in 10-mer PNA)		[51]
10.	RcPNA	Ruthenocene PNA		[52]

TABLE 3 Miscellaneous modifications in PNAs

Sl. no	Abbreviation	Name (expanded)	Structure/detail	References
1.	bis-PNA	bis-PNA	H-TCTCT3-$(egl)_3$-T_3CTCT-LysNH_2 Two PNA segments are connected via a flexible linker composed of multiple units of either 8-amino-3,6-dioxaoctanoic acid (egl) or 6-aminohexanoic acid	[53]
2.	E-OPA	(*E*)-Olefinic polyamide nucleic acids		[43]
3.	F-OPA	Fluorinated olefinic peptide nucleic acid		[54]
4	HypNA-pPNA	*trans*-4-hydroxy-L-proline PNA and phosphonate PNA		[55]
5.	ONA ornPNA	Ornithinyl PNA		[56]

(continues)

TABLE 3 (continued)

Sl. no	Abbreviation	Name (expanded)	Structure/detail	References
6.	OPNA	Oxy-PNA		[57]
7.	OPA	Olefinic polyamide nucleic acids		[43]
8.	PANA	Polyamine nucleic acid		[58]
9.	pHypNAs	Trans-4-hydroxy-N-acetylpyrolidin e-2-phosphonate		[59]
10.	POPNA	Pyrrolidine oxy peptide nucleic acid		[57]
11.	PFP-PNA	Penta fluoro phenyl PNA		[48]
12.	PDC	PNA–DNA chimera		[60,61]
13.	Pmg PNA	*N*-(pyrrolidinyl-2-methyl) glycine PNA		[62]
14.	pPNA PHONA	Phospho-PNA, phosphonic ester nucleic acids		[63]
15.	tPNA	Thioester nucleobase-containing PNA		[64]

to give the corresponding acids, which were readily cyclized to give cyclic Bts PNA monomers (Scheme 1).

PNA-encoded library synthesis suffers from limited choice of reactions due to the special precautions required by protecting group that are not practical. This led to the synthesis of PNA monomers with Mtt/Boc protecting method. Different monomers were synthesized in good yields by monoprotection of ethylene diamine with Mtt-Cl followed by alkylation with

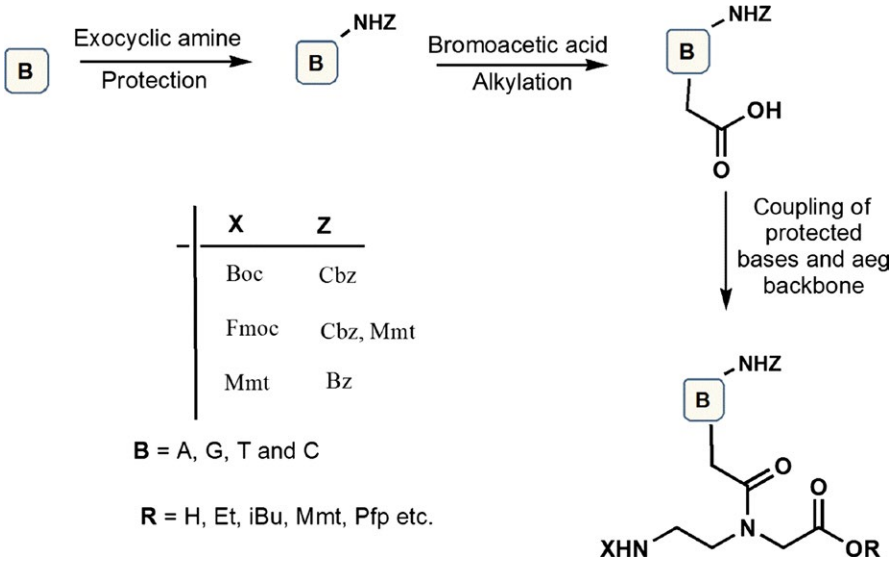
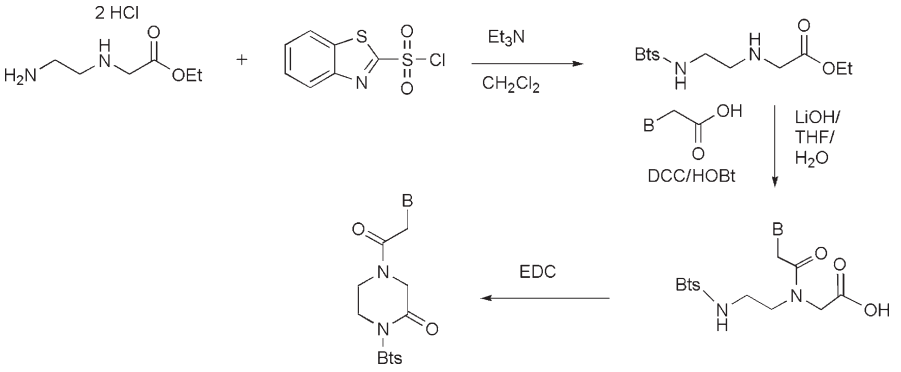


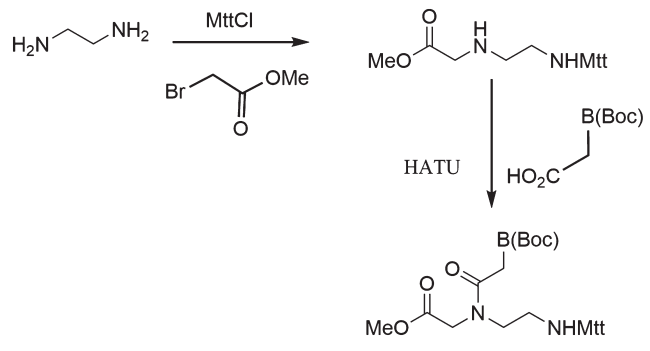
FIGURE 6 General synthesis of PNA monomers



SCHEME 1 Synthesis of Bts PNA monomers

methyl bromoacetate. Coupling of the resultant backbone with Boc-protected nucleobases and subsequent saponification produces the Mtt/Boc PNA monomers (Scheme 2).[77]

1-(4,4-Dimethyl-2,6-dioxacyclohexylidene)ethyl/4-methoxytrityl (Dde/Mmt)-protected PNA monomers are also reported.[78] The Fmoc peptide and Dde–PNA syntheses are especially useful in the synthesis of PNA–peptide conjugates due to mild Dde deprotection and full compatibility to Fmoc chemistry. The alkylation of chloroacetic acid with ethylendiamine gives 2-aminoethylglycine. Dde protection following esterification of aeg yields the PNA backbone ethyl 2-N-Dde-aminoethylglycinate. Couplings of different nucleobase acetic acids were achieved using different reagents as shown in Scheme 3.



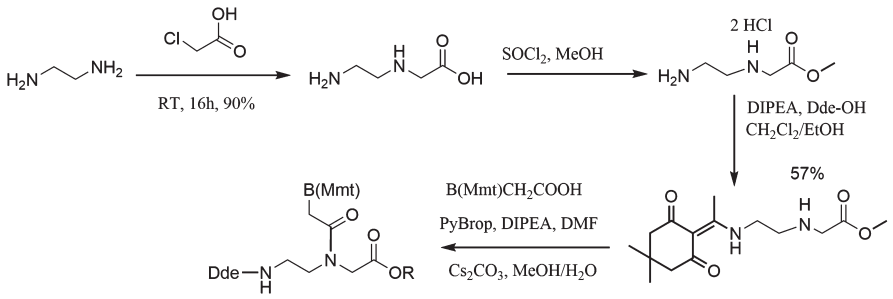
SCHEME 2 Synthesis of Mtt/Boc PNA monomer

3.2 | PNA oligomer synthesis

PNAs can be synthesized manually or by automated processes on solid support or in homogenous solution in high yield and purity, using different orthogonal protecting group strategies (*vide supra*).

3.2.1 | Solid-phase synthesis of PNA oligomer

PNA is synthesized by assembling suitably protected monomers on solid support by well-established standard SPPS.[66] The first report on PNA oligomer synthesis was based upon Merrifield solid-phase synthesis using Boc/Cbz protecting group strategy, which is still applicable today.[14] Subsequently, Fmoc/Bhoc and Mmt/acyl strategies were also developed. Since then, many more different protecting group strategies have been developed based on similar principle. In general, the synthesis of PNA oligomers begins with the monomer loading



SCHEME 3 Synthesis of Dde–PNA monomers

on the polymeric support. The N-terminal protecting group is then deprotected followed by coupling with another monomer. The elongation process consists of several steps of deprotection of N-terminus of the monomer and coupling to the following N-protected monomer. Finally, the oligomer is cleaved from the solid support to give the desired oligomer.

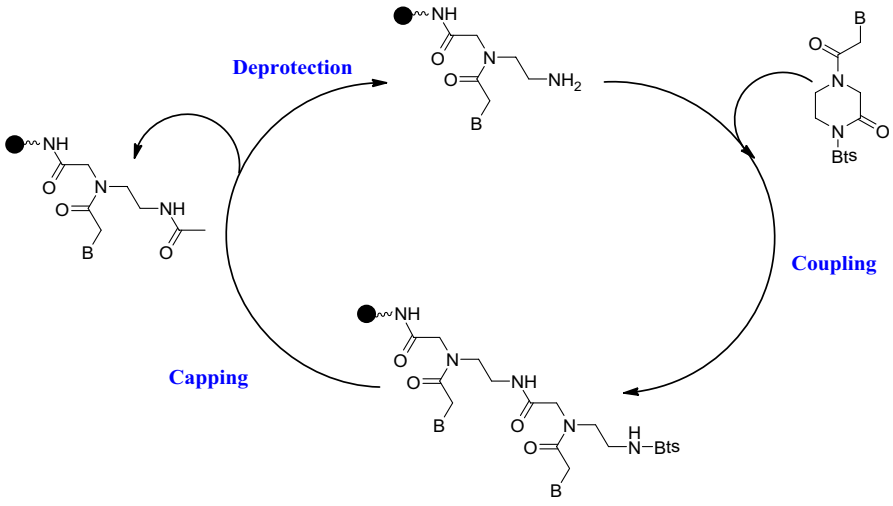
A new benzothiazole-2-sulfonyl (Bts)-protected cyclic monomers was used in the development of newer PNA synthetic method.[76] In addition to a protecting group of the PNA backbone in these PNA monomers, Bts group plays an important role as an activating group for the coupling reaction. The oligomerization process begins with the deprotection. Then, Bts-activated carbonyl of piperazinone is attacked by the primary amine of PNA monomer in the coupling step. The synthesis is completed by capping providing an excellent purity of PNA oligomers (Scheme 4).

3.2.2 | Liquid phase synthesis of PNA oligomer

Liquid phase synthetic strategies are useful in the preparation of short poly PNAs upto decamers.[69] These synthetic strategies allow the use of wider choice of solvents, protecting groups, and reagents for each step. Moreover, they enable coupling of larger fragments. The large-scale PNA synthesis although inexpensive is a tedious process. Liquid phase syntheses are better adapted to functionalize PNA fragments prior to their covalent attachment to various molecules (including peptides, intercalators, steroids, and oligonucleotides), which are useful in increasing the affinity and the specificity of PNAs.

The synthesis of short orthogonally protected PNAs has been reported following convergent and divergent approach.[69] The first strategy was analogous to the classic convergent stepwise approach in peptide synthesis. The PNA moieties are successively elongated, *via* their carboxy end, using a three-step procedure. This procedure consists of condensing the PNA unit with the *N*-2-aminoethyl glycine ester backbone, onto which is then connected the nucleobase acetic acid unit. Hydrolysis generates the carboxy function required for the next elongation step. Thus, a fully protected backbone containing suitable orthogonal protecting group is built. Finally, deprotection and successive attachments of different nucleobases onto the backbone result in the formation of desired PNA.

Liquid phase synthesis of PNA oligomer can also be achieved by divergent approach, that is, of the coupling of two PNA fragments obtained following one of the earlier strategies. For example, a cyclic N-protected hexameric (aminoethylglycinamide) was obtained by coupling suitable orthogonal fragments. Then, a series of selective deprotection and couplings afforded the desired oligomer in good yields.[70] This procedure could be used for the synthesis of short cyclic PNAs containing different nucleobases. At present, solid-phase synthesis is more preferred over solution phase due to ease of synthesis, less time, and easy purification.



SCHEME 4 Solid-phase synthesis of PNA oligomers

3.3 | Postsynthetic processes

Postsynthetic procedure involves cleaving of PNA oligomers from the solid support similar to traditional peptide chemistry. High-performance liquid chromatography (HPLC) is performed at the end of the purification process. Crude PNA is purified by reverse-phase HPLC and characterized preferably by matrix-assisted laser desorption ionization time of flight (MALDI-TOF). The fast atom bombardment (FAB) or electrospray mass spectrometries are also used for characterization.

4 | CHEMICAL AND PHYSICAL PROPERTIES

Over the last two decades, PNA has established itself as an excellent mimic of DNA with remarkable binding properties, extraordinary thermal stability, and unique chemical and physical properties. This section deals with the unique features of PNA, which makes PNA superior than most of other contemporary DNA mimics of its time or earlier.

4.1 | Chemical stability

PNA is chemically stable unlike natural oligonucleotides. PNAs are stable to weak bases; hence, it can be synthesized by Fmoc strategy and various NH_2-cleavable protection group schemes. The chemical instability during the synthesis of PNA oligomers under the basic conditions is reported when there is a free -NH_2 functionality at the N-terminus. Infact, these are more like side reaction than instability, which can be suppressed by capping the free N-terminal and cooperative stabilization effects in PNA/DNA chimeras.[79]

PNAs are completely acid stable, whereas DNA is susceptible to depurination at acidic pH (pH 4.5~6.5). PNAs show great stability against various chemicals and over a wide range of pH and temperature.[32] Hence, PNAs can also be synthesized using acid labile protecting group schemes from peptide chemistry (*vide supra*). Acid labile Boc synthetic strategies are more suitable for PNA synthesis.

4.2 | Thermal stability

Higher thermal stability is another key feature of PNA complexes. Detailed studies have confirmed that the duplexes of PNA with DNA and RNA have higher thermal stability compared to DNA–DNA and DNA–RNA duplexes. The absence of repulsive interactions between the neutral PNA backbone and charged DNA/RNA strand is responsible for the stronger binding effect. Convincing results obtained from the studies based on thermal stability of PNA-based duplexes in aqueous solution reveal that the melting temperature (Tm) per base pair of a PNA/DNA duplex is 1 °C higher than that of corresponding DNA–DNA duplex.[80]

Binding Properties. PNA shows stronger binding properties independent of the ionic strength of the medium, due its charge-neutral backbone. The Tm values of PNA–DNA duplexes are independent of the salt concentration, whereas the stability of DNA–DNA duplexes decreases at low ionic strength.[32] PNA binds to corresponding complementary sequence even in the absence of Mg^{2+}. The stability of PNA–DNA duplex decreases only slightly with increasing Na^+ concentration due to counterions released upon duplex formation. PNA has strong sequence-specific hybridization affinity toward both natural and synthetic oligonucleotides to form Watson–Crick structures. Mixed-base PNAs hybridize with complementary single-stranded DNA/RNA or even with PNA to form duplexes. The hybridization is so specific that even single base pair mismatch in the hybrid complex may lower the Tm by 8–20 °C. These PNA/DNA and PNA/RNA duplexes show higher stability due to lack of electrostatic repulsion with the negatively charged DNA and RNA. On the basis of melting temperature (Tm) analysis, the stability of these duplexes can be generalized as follows: PNA–PNA > PNA–RNA > PNA–DNA > DNA–DNA > DNA–RNA.

Interesting results are also obtained when PNA interacts with double-stranded DNA. The conventional (PNA/DNA_2) triplex is not formed by thymine rich PNA; rather, it forms strand displacement complex $(PNA)_2$-DNA and results in displacement of second DNA strand into a 'D' loop.[81] One PNA strand is stabilized through Watson–Crick bonding while the other strand forms Hoogsteen base pairs in these triplexes. To enhance strand invasion efficiency, major groove triplex binding and sequence selective recognition, PNA clamps or bis-PNAs (two homopyrimidine PNA oligomers covalently connected by a flexible linker) are generally used resulting in the formation of locally looped PNA structures known as P-loop.[82] The displaced ssDNA can further hybridize with a ssDNA to form PD-loop, which consists of locally open dsDNA, a pair of bis-PNA 'openers', and an oligodeoxyribonucleotide.[83] Similarly, PR- and PP-loops can be formed by PNAs in which a substituted oligoribonucleotide or PNA, respectively, is used.

4.3 | Solubility

In general, PNAs have reasonable water solubility, but this is strongly dependent on pH and the buffer used. PNAs with higher than 60% G-rich purine particularly longer than 12 units may show solubility problems. PNA also shows poor aqueous solubility in phosphate buffers.[84] Cysteines are incorporated at N-terminal to conjugate the peptides via disulfide coupling. Further, incorporation of positively charged lysine residues at the carboxyl-terminal, O-linker or other solubility-enhancing molecules, backbone modification, and incorporation of

negative charges in the PNA–DNA chimeras enhances the solubility of PNA oligomers in both organic as well as in aqueous solvents.[85,86] All such modified PNAs and many more that are even not mentioned here can be obtained commercially.

5 | BIOLOGICAL PROPERTIES

PNAs are resistant to enzymatic degradation extending their lifetime both *in vitro* and *in vivo*. The peptide backbone of PNAs is not easily recognized by nucleases and proteases. Consequently, it is found to be biostable in human serum, bacterial cell extracts, nuclear mouse ascites, tumor cell extract, isolated fungal proteinase K, and peptidase.[87] It is well established that PNA has the potential to inhibit replication and induce transcription arrest and translation arrest. Several good reviews have been published which discuss the antisense and antigene properties of PNA extensively.[88] An antigene PNA binds to complementary sequence in DNA, while antisense PNAs hybridize to specific mRNA and inhibit translation (Figure 7). Furthermore, they may also activate

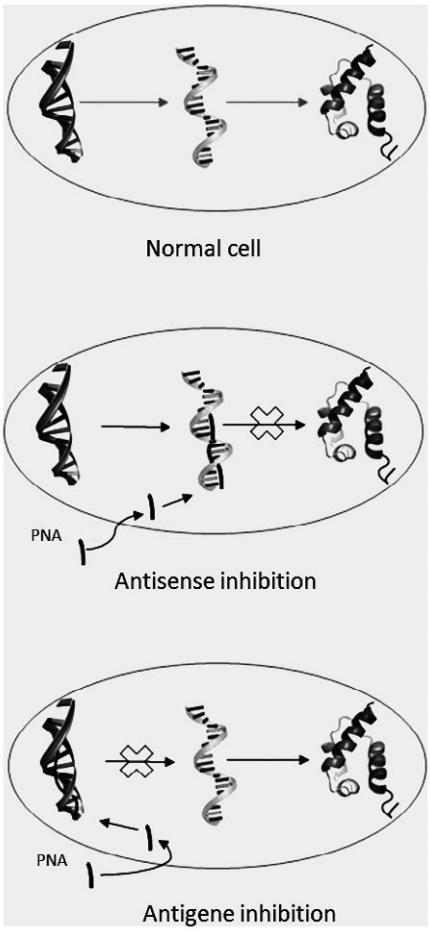


FIGURE 7 Antisense and antigene properties of PNA

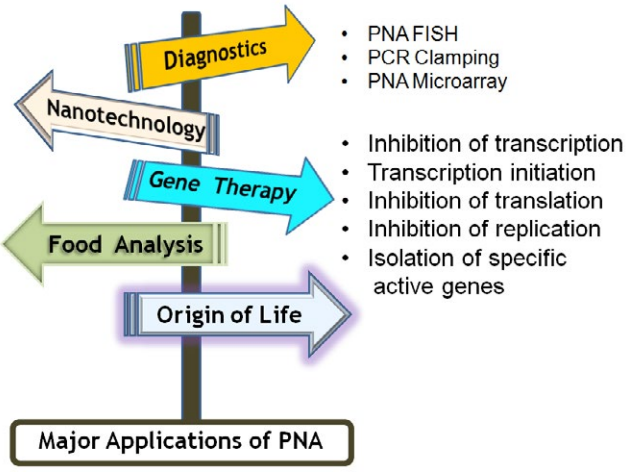


FIGURE 8 Major applications of PNA

transcription artificially. PNAs are non-immunogenic. The studies directed to evaluate the antigenicity revealed that PNAs are unable to elicit an antibody response when injected alone.[89] However, they can be made immunogenic by linking to a protein immunogenic carrier.[90] These properties place PNA at a great potential advantage as a therapeutic compound. However, the cellular uptake of PNA is very slow (*vide infra*), which is the major limitation that should be overcome before it can be used for the biological use. Thus, if the issues related to uptake or cellular delivery is solved, PNA has favorable biological properties for the development of gene therapeutic drugs. Nonetheless, its biological properties are proving beneficial in developing diagnostic tools and biochemistry.

6 | CELLULAR DELIVERY

The intracellular delivery of this unique nucleic acid analog is very poor or negligible, which is one of the major challenges that must be overcome before it can be used for therapeutic purposes. Various approaches for cellular delivery of PNA include use of cell penetration peptides, microjection, electroporation, use of cationic lipids, cotransfection with DNA, permeabilization, direct delivery, etc. Studies revealed that coupling of PNA to different carriers improves their uptake into cells. Among these, several peptide sequences have been shown to be able to carry PNA oligomers across the cell membranes. For example, conjugation of PNAs to natural proteinous ligand was used by Tsutoma *et al.* as a nontoxic vector for cellular delivery. He designed asialofetuin conjugates with DNA (AF/DNA) for cell-specific delivery and demonstrated that intravenous injection into mice can deliver PNAs to liver. These PNAs delivered to hepatic cells showed antisense effects by receptor-mediated endocytosis.[91] A controllable and reversible method for the creation

of PNA-based nanocomposites capable of penetrating cell membranes is also reported.[92]

Electroporation can also be used as an effective approach for CPP-based PNA delivery and bacterial inhibition by creating small pores in the membrane. Difficulty in the endosomal escape limits the effective killing of bacteria inside macrophages (mammalian cells). Electroporation delivers the molecules by eliminating endocytosis (cargo encapsulation inside endosomes) and significantly enhances the bioavailability of CPP–PNAs. Recent reports show that the delivered $(KFF)_3$K-O-PNA in complex cellular systems possesses increased antisense effect and kills Salmonella *entericaserovar Typhimurium* LT2 inside macrophages.[93] Dedicated efforts by several groups may yield a promising future for peptide nucleic acids drug delivery system.

7 | APPLICATIONS

PNA, due to its superior properties than other DNA mimics, has attracted wide attention among chemists and biologists offering promises and new challenges to explore multitude of studies toward the development of a gene therapeutic drug. It has surprisingly led to the development of antisense and antigene technologies, various biomolecular tools including nucleic acid biosensors, tools for genome mapping, hybridization techniques for genetic detection, modulation of PCR analyses, FISH technology, and so forth (Figure 8).

7.1 | Gene therapy

The therapeutic potential of PNA for drug development was anticipated from the beginning. Its unique properties such as higher affinity, strong specificity for the targeted sequences, and no toxicity inside cell even at high concentration make it a strong candidate for the design and development of gene therapeutic drug.

The general strategies reported to design the PNA-based therapy are as follows:

- Inhibition of transcription
- Artificially initiate transcription
- Inhibition of translation
- Inhibition of replication
- Isolation of specific active genes

The proof of principle for the use of PNA as antisense and antigene therapy is well established. Its use is hampered due to the poor solubility in water and low cell permeability. Tremendous research work directed in this direction is carried worldwide *albeit* the progress is somewhat slow.

PNA offers ample opportunity in the development of novel therapeutic approaches. It has been recently shown that specific RNA targeting using antisense PNA molecules can efficiently manipulate gene expression in *P. falciparum*. Kolevzo and coworkers <for the first time demonstrated treating malaria infections using PNA technology.[94] They successfully achieved specific downregulation of *P. falciparum* gene expression by conjugating antisense PNA with octa-D-lysine CPP. Hence, it may offer an alternative approach to silence the essential genes and produce antimalarial activity without any fear of emergence of drug resistance which is widespread in conventional small drug molecules.[94,95] Antisense targeting of pre-mRNA splicing offers a promising approach for discovery of anticancer drugs as well. Several PNA 9-aminoacridine conjugates have been identified which can target the 5′- or 3′-splice sites in intron 2 or the 3′-splice site of intron3 of mdm 2 and inhibit pre-mRNA splicing of the human cancer gene in JAR cells.[96] PNA-based microRNA inhibitors also demonstrated anti-inflammatory effects by suppressing neuroinflammation in microglia cells.[97] The ability of PNA to inhibit pathogenic bacteria is also reported. Studies demonstrate that anti-dnaK PNAs are mildly effective on E. coli and very effective on S. enterica strains *in vitro*.[98] The growth of bacterial cultures is also inhibited *in vitro* by CPP–PNA molecular systems. Further, it was found that electroporation enhances the bioavailability of $(KFF)_3$K-O-PNA and inhibits intracellular Salmonella inside macrophages.[99]

We have also reported Bactericidal antisense effects of peptide–PNA conjugates. We showed 9- to 12-mer PNAs, attached to the peptide KFFKFFKFFK, show enhanced potency in *E. coli*.[109] Further, antimicrobial peptide–PNAs kill pathogenic bacteria selectively without targeting other species. PNA identifies the difference in species in the translation initiation region of essential genes and selectively inhibits the growth of *E. coli*, *B. subtilis*, *K. pnuemoniae,* and *S. enterica serovar Typhimurium* in axenic or mixed culture.[102] Some selected most recent publications of PNA in antisense and antigene therapy are summarized in Table 4.[93,95,100–108]

7.2 | Nanotechnology

PNA supersedes DNA and has also received significant attention in recent years for its application in nanotechnology. It has the ability to organize nano-objects into desired architectures for the functional exploitation of unique properties of nanomaterials. PNA nanoparticles are promising self-assembling systems. They are used as the connector for the assembly of DNA-based nanostructures. Proteins and amino acid complexes can also be assembled into a 3D DNA nanocage using a PNA linker to control the assembly and rationally design nanoscaffolds for protein and polypeptide engineering.[110,111] Moreover, PNA probes

TABLE 4 Some recent antisense and antigene applications of PNA

Application	Description	References
Regulating var gene activation	Antisense long non-coding RNAs regulate var gene activation in the malaria parasite *Plasmodium falciparum*	[95]
Exon skipping enhancement	Peptide conjugates of phosphorodiamidate morpholino oligonucleotide showed enhanced activity in an exon skipping assay in Duchene's muscular dystrophy using skeletal mouse mdx cells	[100]
Regulation of gene expression	PNAs can inhibit miR-509-3p, cystic fibrosis transmembrane regulator and disease gene of cystic fibrosis	[93,101]
Silencing of the B-cell lymphoma 2 (Bcl-2) protein expressions	Antisense PNA by conjugation to fluorescent mesoporous silica nanoparticles. PNA-conjugated mesoporous silica nanoparticle was endocytosed by HeLa cancer cells and released PNA into cancer cells inducing effective silencing of the B-cell lymphoma 2 (Bcl-2) protein expression	
Antimicrobial peptide–PNAs	Species-selective PNA antibacterials can selectively inhibit growth of *B. subtilis*, *E. coli*, *K. pnuemoniae,* and *S. enterica serovar Typhimurium* in axenic or mixed culture	[102]
PNA functionalized cationic nanocomplex for in vivo mRNA detection	The early diagnosis of acute lung injury was enabled by electrostatic complexation of a radio-labeled antisense PNA-YR9: oligodeoxynucleotide (ODN) hybrid and a cationic nanoparticle (cSCK). The nanocomplex efficiently entered RAW264.7 cells and efficiently detected iNOS mRNA *in vitro* and *in vivo*	[103]
Antibacterial antisense PNA	Peptide–PNA conjugates targeted to the translation initiation region of the acpP and ftsZ gene of P. aeruginosa (PA01) can completely inhibit P. aeruginosa strains LESB58, PA14, and PA01 at 1- to 2-mM concentrations	[104]
Cellular inhibition of miR-122	Efficient miR-122 inhibition was obtained with cationic PNAs at sub-micromolar concentrations. PNA anti-miRs were able to sustain miR-122 inhibitory effects	[105]
PNA-based artificial nucleases	Artificial nucleases composed of a PEG–PNA–PEG domain conjugated to HGG·Cu and DETA as well-known cleavage sites act as potential antisense and anti-miRNA agents	[106]
Local and systemic dystrophin splice correction	Long-term splice correction of the *DMD* gene in *mdx* mice was obtained by intramuscular PNA delivery and effective splice correction in aged *mdx* mice	[107]
Efficient inhibition of miR-155 function *in vivo*	MiR-155 inhibition by PNA in primary B cells was achieved in the absence of any transfection agent. Interestingly, PNA also induced additional changes in gene expression	[108]

can also be incorporated into the nanochannel to construct sensing platform for the detection and identification of DNA molecules.[112]

Sequence-specific recognition of DNA can be achieved using PNA/DNA hybridization in nano confined environment of modified synthetic ion channels. PNAs tagged with four lysine residues can be developed as amperometric genosensors. The use of a nanostructured surface as the substrate for the sensor assembly results in the selective recognition of the target oligonucleotide sequence with high sensitivity.[113] Similarly, anion-exchanged nanosolid support with poly((2-diethylamino)ethyl methacrylate)-grafted cationic magnetite nanoparticle was used for the detection of DNA sequences by employing PNA probe.[114] Peptide nucleic acids also find application as reversible switches in a DNA rotaxane architecture. Pseudo-complementary PNA actuators can be combined with both light and toehold-based switches. Orthogonal switching approaches for DNA architectures may open up new avenues in dynamic DNA nanotechnology.[115]

Notably, PNA–AuNP hybrids can be prepared without altering PNA functionality. The Au(111) surface has been widely used in biosensor applications due to strong gold–sulfur (thiol) interactions between the bifunctional linker molecules (cysteamine (thiol–amine terminated) or 1,4-benzenedithiol (thiol–thiol terminated)) and the substrate surface. Strong interactions between the surface of the AuNP and the nitrogen atom of the amine group of cysteamine (or sulfur atom of thiol group of 1,4-benzenedithiol) were exploited to attach the AuNPs onto the linker-modified Au(111) surface. It was then subsequently functionalized with the ssPNA probes. Interestingly, PNA immobilized onto the surface-affixed gold nanoparticle (AuNP)-modified Au(111) does not lead to aggregation and exhibit remarkable target detection ability.[116] On the other hand, selective aggregation induced by the electrostatic attraction of the PNA-modified AuNPs is also exploited in the colorimetric detection of c-Kit mutations.[117] Further, DNA-mediated AgNP growth on a PNA-modified glass slide was exploited for the construction of label-free surface-enhanced Raman spectroscopy (SERS) method for DNA analysis with a detection limit of 45 pM. The hybridization of PNA with DNA makes the surface negatively charged and facilitates the absorption of silver ions on the DNA skeleton. Silver nanoparticles were then formed by chemical reduction. Absorbing rhodamine 6G on AgNP surface leads to further growth of AgNPs and amplification of the detectable SERS signals.[118]

Another application of PNA in nanotechnology is its role as miRNA sensor with the detection limit in picomolar. The PNA-functionalized carbon nitride nanosheets have also been used as a probe for *in situ* monitoring of intracellular microRNA sensing for recognition of both cancer cells and intracellular microRNA.[119] The miRNA sensor was also developed from the nanosized graphene oxide (NGO) with PNA probes for quantitative monitoring of target miRNA in living cells. The tight binding of NGO with peptide nucleic acid quenches the fluorescence of the dye conjugated to PNA, and the addition of target miRNA results in the recovery of the fluorescence.[120] The GO nanomaterial combined with a PNA probe has also been reported for sensitive and selective detection of DNA.[121] Xuan *et al.* have demonstrated an ultrasensitive and selective electrochemical nucleic acid sensing method with detection limit down to 100 fM based on the kinetically controlled dendritic DNA/PNA nanostructures assembly. A cascade of toehold-mediated strand displacement reactions assembled the FcPNA, target sequence, and DNA strands on the dendritic nanostructures for sensing application.[122]

Recently, triplex-forming PNAs and donor DNA were used in biodegradable polymer nanoparticles to correct F508del cystic fibrosis transmembrane conductance regulator mutation in human bronchial epithelial cells. Biodegradable PLGA nanoparticles with PBAE and surface-modified with the nuclear-localization sequence-containing CPP MPG were used for PNA delivery. The PNA/DNA-encapsulating PLGA/PBAE/MPG nanoparticles showed enhanced efficacy to achieve site-specific gene modification at the order of magnitude unprecedented in PNA technology.[123] The composites of PNAs with titanium dioxide nanoparticles have also been prepared for therapeutic application. Titanium dioxide nanoparticles coated with polylysine and immobilized DNA/PNA duplexes were used to efficiently deliver PNA molecules which were reversibly immobilized to the carrier with the controlled desorption rate. These nanocomposites penetrate through cell membranes and exhibit antisense activity without significant toxic effects on the living cells. These nanobiocomposites showed better activity against influenza A virus than by same sequence of PNA–Tat conjugate at the same concentration.[124]

7.3 | PNAs in food analysis

PNA can also be used in the food analysis for food authentication, that is, determination of ingredient authenticity by the detection of genetically modified organisms and assessment of the presence of hidden allergenic ingredients and microbial pathogens.

The identification of food-borne diseases and monitoring of GMOs in foods requires considerable attention. PNA can be helpful in developing suitable tools to detect and identify GMOs. Reports were published on the identification of peanut DNA in food at the nanomolar level by using a PNA probe complementary to a tract of the peanut Ara h 2 gene based on circular dichroism studies.[125] Andrea Germini reported detection of genetically modified soybean using PNA microarray technologyfor their safety as food.[126] Another useful tool in the analysis of GMO is PCR clamping induced by PNA. It can provide an unequivocal identification of the presence of transgenic material for legal or commercial consequences. Further, PCR-amplified GMOs DNA can be identified by PNA probes in anion-exchange chromatographic analysis. The presence of transgenic materials in food is assessed not only for the diagnostic application, but also for biomedical research and application in food science. A duplex PCR method was able to specifically detect traces of hazelnut and peanut in 50 pg of their target DNA raw materials and commercial products.[127] PNA probes have also been successfully used for the ultrasensitive detection of genetically modified target using a nanoparticle-enhanced SPRI detection. This method enables to identify zM concentrations of GM target sequence from aM concentration (10–30 pg/L) of GM and GM-free genomic DNA.[128] Recently, PNA molecular beacons were also used for the detection of GM Roundup Ready soybean and single-strand 13-mer sequences in the *O. europaea L. (cultivar Ogliarola)* genomes.[129]

PNA probes may also help to prevent and reduce the incidence of bacterial diseases. They prove to be a highly specific and rapid genotypic means for the detection of Gram-positive bacteria *Listeria* spp. and routine monitoring of food production environments to control *L. monocytogenes*.[130] Use of PNA-FISH is simple, rapid, and advantageous for the detection of *C. coli* from drinking water containing mixed species and identification of thermo-tolerant *Campylobacter spp.* from cultured bacteria. Detection of *C. coli* can be readily achieved at a concentration of 100 cfu/mL water by membrane filtration method from tap water in any laboratory equipped with an epifluorescence microscope.[131]

7.4 | PNA in diagnostics

Peptide nucleic acid has been extensively used as a diagnostic tool for the detection of various diseases. Alzheimer's disease is related to single-nucleotide polymorphisms (SNP), which is a major source of genetic variation in the human genome. Guo *et al.* demonstrated an electrochemical approach to detect single nucleotide mismatch using PNA in *apoE* 4 related to Alzheimer's disease. Electrochemical impedance spectroscopy was used by exploiting the interactions of PNA–DNA films with Ni^{2+} on gold electrodes. The detection limit of 10 fM under *in situ* hybridization conditions was achieved.[132] Rahman *et al.* developed label free colorimetric method for dengue virus detection using PNA probe. The PNA/DNA hybridization using unmodified AuNPs occurs

directly in solution. Aggregation of unmodified AuNPs undergoes immediately due to coating of neutral charged PNA on AuNPs surface. The PNA probe forms a negatively charged complex with the target DNA which results in the dispersion of AuNPs and stability in solution. The immediate color change in this method can be detected by UV–Vis absorption spectra or even by naked eye.[133] Fluorescence detection of KRAS2 mRNA hybridization in lung cancer cells was also carried out with PNA–peptides containing thiazole orange.[134]

7.4.1 | PNA-FISH

Fluorescence *in situ* hybridization is a widely used method in clinical diagnostic laboratory for identification and quantification of micro-organisms in a wide range of samples. The specificity of DNA/rRNA hybridization for the detection of selected bacterial species is combined with the microscopic observation or morphologic visualization. The PNA-FISH method has been used in laboratories for over a decade now for identification of a variety of organisms. It is the most advanced technology for applications in FISH. The availability of diagnostic tests such as those developed by AdvanDx Inc. indicates the robustness and usefulness of this method. They can be integrated into the laboratory as part of routine blood culture work up without difficulty.

The PNA-FISH method was successfully employed for the detection of catheter-related bloodstream infections. The PNA-FISH method as compared to the traditional bacteriological method saves atleast two days in the detection of *E. coli* serotype O157.[135] The clinical consequences of using PNA-FISH in *Staphylococcal* bacteraemia provide fast information on findings in blood cultures.[136] Another report on the comparative analysis of Gram's stain, PNA-FISH, and Sepsityper™ with MALDI-TOF MS was published for the identification of yeast directly from positive blood cultures. The identification of Sepsityper™ with MALDI-TOF MS was although accurate but needs increased sensitivity for successfully identifying yeast commonly encountered in fungaemia. PNA-FISH YTL demonstrated a high success rate and reduced time in the identification process.[137]

FISH method using peptide nucleic acid probes have also been reported for the rapid detection of *Stenotrophomonas maltophilia* from common bacterial species in the respiratory tract,[138] and *candida sp.* from blood and peritoneal fluid specimens.[139] Clarithromycin resistance of *Helicobacter pylori* smears[140] in gastric biopsy specimens can also be diagonized by PNA-FISH.[141] It is also effective in detecting urinary tract infections caused by *Proteus spp.*,[135] *Lactobacillus spp.*, and *G. vaginalis* species in the vagina,[142,143] yeast from 54 clinical isolates of yeasts inoculated into blood culture bottles,[144] directly from a total of 176 blood cultures positive for yeasts at direct Gram stain and 24 negative blood cultures as control collected from 15 Italian hospitals.

A modified second-generation PNA-FISH method developed for saving time with new probe designs and built-in controls is known as QuickFISH. It enables identification of species in 20–25 min based on the fluorescent color of the cells without the need to wash or mount slides. Rapid detection of *Enterococcus spp.* was reported direct from blood culture bottles using *Enterococcus* QuickFISH Method.[145] The *Staphylococcus Quick*FISH method is used for the simultaneous identification of coagulase negative *Staphylococci* and *Staphylococcus aureus* from the blood culture in <30 min. The simultaneous identification of *S. aureus*/CoNS with Gram stain by this quick and expedient method improves the accuracy of therapeutic intervention. A recent report on Quantitative Fluorescence *in Situ* Hybridization (Q-FISH) Method for estimation of telomere length in different cell types of tissue section givesoutstanding results. Telomeres are located at the ends of eukaryotic chromosomes. They get shortened with aging or different reasons. Analysis of the telomeres of individual chromosome is important because the shortened telomere plays significant role in chromosomal instability resulting in carcinogenesis and many diseases related to aging.[146] Earlier analysis of telomeric DNA in mitotic and meiotic chromosomes of five opisthorchid species, viz. *Opisthorchis felineus*, *Opisthorchis viverrini*, *Metorchis xanthosomus*, *Metorchis bilis,* and *Clonorchis sinensis,* was also reported using PNA-FISH method.[147]

7.4.2 | Polymerase chain reaction

Polymerase chain reaction (PCR) clamping using peptide nucleic acid blocking primer may be used to selectively amplify target DNA for the development of various analysis techniques. The PNA–PCR clamping technique was applied to investigate the community structures of rhizobacteria associated with plant roots. PCR can easily amplify small subunit ribosomal RNA (SSU rRNA) genes from spinach and cucumber roots with a set of universal primers for bacteria. Selective amplification of bacterial genes can then be obtained by suppressing the PCR amplification of the organelle SSU rRNA genes.[148] PNA-mediated PCR clamping allows the selective inhibition of the ERVWE1 gene amplification. Recently, QPCR technique was developed for the quantification and detection of the multiple sclerosis-associated retrovirus belonging to the human endogenous retrovirus-W family.[149] PNAs were established as both PCR clamp and sensor probe for the detection of low copies of drug-resistant influenza viral gene. A rapid and sensitive method was developed to monitor the emergence of a drug-resistant virus during the treatment of infected patient. Earlier procedure for sequencing the resistant markers from the viral isolate takes several days; however, PNA-mediated PCR technique takes

only few hours. PNA–PCR clamping technique has also been used in the detection of EGFR mutations in the circulating free DNA,[150] *BRAF* V600E Mutation with Thyroid Tissue,[151] *KRAS* mutations in peripheral blood of colorectal cancer patients,[152] and T790M Epidermal Growth Factor Receptor Mutation.[153] The natural diets of larval marine animals and Japanese eel leptocephali were also investigated using PNA-directed PCR clamping.[154]

7.4.3 | Microarray

Microarray technology is used in diagnosis to provide accurate and high throughput parallel analysis. It consists of a large number of functionalized probes immobilized on a solid substrate. The sequence-specific and selective hybridization properties of PNA have great potential in the development of PNA-based microarrays. PNA is used in many nucleic acid based biosensors with enhanced detection sensitivity. The large number of PNA probes can be immobilized on a small substrate (glass, Au, silicon, etc.) in a PNA microarray for fast, highly sensitive, and high-throughput analysis. The PNA microarray technology finds application in pathogen detection, single-nucleotide polymorphism (SNP) detection, and monitoring of disease-related miRNA expression. The readers are referred to a recent review, which discusses the fabrication methods, various detection methods, and current applications of PNA microarrays.[155]

8 | ORIGIN OF LIFE

PNA might also address the persistent curiosities and inevitable queries of human mind to unravel the mystery of emergence and existence of life on earth. Life as we know in the present-day organisms has a central mechanism which is built up around the same set of biomolecules DNA, RNA, and proteins, abiding by the central dogma of molecular biology.[1] However, there are no evidences to make us believe that life existed in its present form from the beginning. Many researchers supported the concept of RNA world theory, according to which RNA was assumed to serve as both enzymes and genetic storage molecule.[156] The discovery of catalytic and self-replicating RNA molecule brought wide acceptance to this theory. However, high sensitivity and instability (hydrolysis) of RNA along with the difficulty to produce ribose sugars or ribonucleosides in primitive conditions suggests that the original replicators may have been a related kind of molecules without the ribose phosphate backbone or a totally different molecule.

The synthesis of PNA[14] offered new hope in the studies related to origin of life.[157] Miller and Oro had already synthesized simple amino acids[158] and purine nucleobases[159], respectively, by creating laboratory conditions similar to the primordial earth, confirming the possibility of formation of fundamental building blocks of life in prebiotic conditions. Further, Nelson *et al.* synthesized some components of PNA under simulated prebiotic conditions.[160]

The presence of diaminobutyric acid, a building block for PNA, and its analogs in Murchison meteorite[161] also suggests that PNA might have preceded RNA. Recent reports on PNA self-replicating system analogous to biological replication,[162] Keidrowski's work on short self-complementary PNA sequence capable of promoting autocatalytic self-replication,[163] and Leslie E. Orgel and coworker's convincing report on the genetic transition between PNA and RNA[164] without any loss of information strengthen the speculations on the role of PNA in origin of life (Figure 9).

PNA also gives a plausible path of origin of homochirality. Homochiral molecules might have evolved from PNA (nonchiral polymer), which exists as an equal left- and right-handed duplex, and chirality can be induced by an amino acid attached to the end of one of the PNA strands.[165] Thus, there are strong reasons to believe that in the prebiotic earth conditions, PNA came first and eventually transformed into RNA with the introduction

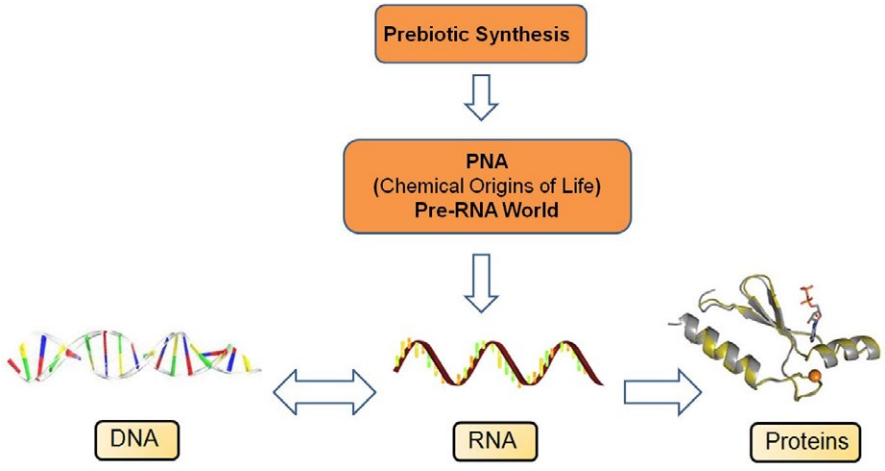


FIGURE 9 Schematic representation of plausible role of PNA in origins of life.

of chirality. Notwithstanding more experimental proofs which are required to validate this line of thought, PNA provides some fascinating insights into the study of origin of life.

9 | CONCLUSIONS

The goal of PNA discovery was to design new DNA mimics purely based on triplex formation with complementary DNA to target gene functions. Although the PNA-based gene therapeutic drug has not reached drug store yet, we are way ahead of where we were two and half decades ago and the field has moved to a point of maturity. PNAs may also provide valuable insights into the emergence and evolution of alternative forms of life. It may give new ideas to unexplore methods of storing and transmitting genetic information along with the development of various molecular diagnostics tools. PNA reigns superiority to its natural counterparts and has clear advantages with high binding affinity and strong specificity despite relatively poor or negligible solubility and tendency for self-aggregation in aqueous medium. Since its discovery, synthetic chemists have extensively modified its structure with mixed success. Mostly, the focus was on the modifications of backbone and heterocyclic nucleobase. Targeted structural modifications have extended the arsenal of various biotechnological and diagnostic tools. We believe that the use of chemical approaches to tailor cationic modifications, for example, preparation of PNA–CPP conjugates or development of GPNAs, may improve the essential properties of PNAs that would make it more resourceful.

Judging from the expanding literature in this field, it is just to conclude that although the primary goal of creating a PNA-based drug is unmet, harnessing its potential to make a new generation of drugs remains the challenge for the next decade. PNA is easy to synthesize and metabolically stable, yet there are only few reports of convincing biological activity, whether antigene, antisense, antiMiR, or splice control is generally sparsely used compared to other technologies. A critical analysis of this conundrum from the continuously expanding use of PNA in biomedical applications and diagnosis may yield remarkable insights.

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CONFLICT OF INTEREST

There is no conflict of interest among the authors.

ABBREVIATIONS

A, adenine; AgNP, silver nanoparticles; AuNP, gold nanoparticles; Bcl, B-cell lymphoma; Boc, tert-butyloxycarbonyl; Bhoc, benzhydryloxycarbonyl; Bts, benzothiazole-2-sulfonyl; C, cytosine; CBz, carboxybenzyl; CFU/mL, colony-forming units per milliliter; CPP, cell penetrating peptide; DCC, *N,N′*-Dicyclohexylcarbodiimide; Dde, 1-(4,4-dimethyl-2,6-dioxacyclohexylidene)ethyl; DETA, diethylenetriamine; DNA, deoxyribonucleic acid; ds, double stranded; FAB, fast atom bombardment; FISH, fluorescence *in situ* hybridization; fM, femtometer; Fmoc, fluorenylmethyloxycarbonyl; G, guanine; GM, genetically modified; GMO, genetically modified organisms; GO, graphene oxide; HOBt, hydroxybenzotriazole; HPLC, High-performance liquid chromatography; L, litre; MALDI-TOF, matrix-assisted laser desorption ionization time of flight; MBHA, methylbenzhydrylamine; miR, microRNA; Mmt, methoxytriphenylmethyl; Mtt, 4-methyltrityl; NGO, nanosized graphene oxide; PBAE, poly beta amino ester; PCR, polymerase chain reaction; PEG, polyethylene glycol; pg, Pictograms; PLGA, poly(lactic-coglycolic) acid; pM, picometer; QPCR, quantitative PCR; RNA, ribonucleic acid; SERS, surface-enhanced Raman spectroscopy; SNP, single-nucleotide polymorphism; SPPS, solid-phase peptide synthesis; ss, single strandedssss; SSU, small subunit ribosomal; T, thymine; UV–Vis, ultraviolet–visible; YTL, yeast traffic light.

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