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Pyrrolidinyl PNA with α/β-Dipeptide Backbone: From Development to Applications

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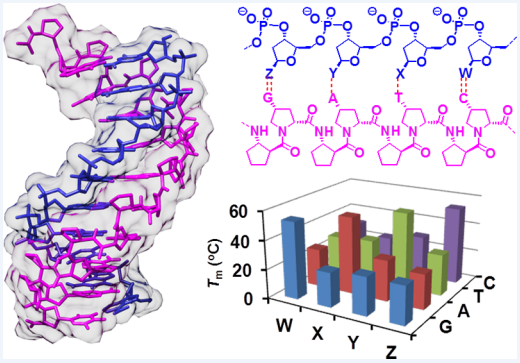
CONSPECTUS: The specific pairing between two complementary nucleobases (A·T, C·G) according to the Watson−Crick rules is by no means unique to natural nucleic acids. During the past few decades a number of nucleic acid analogues or mimics have been developed, and peptide nucleic acid (PNA) is one of the most intriguing examples. In addition to forming hybrids with natural DNA/RNA as well as itself with high affinity and specificity, the uncharged peptide-like backbone of PNA confers several unique properties not observed in other classes of nucleic acid analogues. PNA is therefore suited to applications currently performed by conventional oligonucleotides/analogues and others potentially beyond this. In addition, PNA is also interesting in its own right as a new class of oligonucleotide mimics. Unlimited opportunities exist to modify the PNA structure, stimulating the search for new systems with improved properties or additional functionality not present in the original PNA, driving future research and applications of these in nanotechnology and beyond. Although many structural variations of PNA exist, significant improvements to date have been limited to a few constrained derivatives of the privileged *N*-2-aminoethylglycine PNA scaffold. In this Account, we summarize our contributions in this field: the development of a new family of conformationally constrained pyrrolidinyl PNA having a nonchimeric α/β-dipeptide backbone derived from nucleobase-modified proline and cyclic β-amino acids. The conformational constraints dictated by the pyrrolidine ring and the β-amino acid are essential requirements determining the binding efficiency, as the structure and stereochemistry of the PNA backbone significantly affect its ability to interact with DNA, RNA, and in self-pairing. The modular nature of the dipeptide backbone simplifies the synthesis and allows for rapid structural optimization. Pyrrolidinyl PNA having a (2′*R*,4′*R*)-proline/(1*S*,2*S*)-2-aminocyclopentanecarboxylic backbone (acpcPNA) binds to DNA with outstanding affinity and sequence specificity. It also binds to RNA in a highly sequence-specific fashion, albeit with lower affinity than to DNA. Additional characteristics include exclusive antiparallel/parallel selectivity and a low tendency for self-hybridization. Modification of the nucleobase or backbone allowing site-specific incorporation of labels and other functions to acpcPNA via click and other conjugation chemistries is possible, generating functional PNAs that are suitable for various applications. DNA sensing and biological applications of acpcPNA have been demonstrated, but these are still in their infancy and the full potential of pyrrolidinyl PNA is yet to be realized. With properties competitive with, and in some aspects superior to, the best PNA technology available to date, pyrrolidinyl PNA offers great promise as a platform system for future elaboration for the fabrication of new functional materials, nanodevices, and next-generation analytical tools.



1. INTRODUCTION

Recognition between two complementary nucleic acids via highly specific Watson−Crick base pairing is the basis for the storage and transfer of genetic information. The high fidelity of the recognition event suggests potential uses of nucleic acids for regulation of genetic expression, as a probe for nucleic acid sequence detection, and many other purposes.[1] In addition, the Watson−Crick base pairing allows one to program nucleic acids to fold into complex architectures in a controllable fashion, making them particularly attractive as building blocks or scaffolds for the creation of novel structures or materials exhibiting unique functions.[2−4] However, natural nucleic acids are not always ideal candidates for these "unnatural" purposes because of their limited stabilities and only modest binding affinities. Fortunately, the backbone of nucleic acids can tolerate structural modification to some extent without sacrificing the ability to form Watson−Crick base pairs. Such modified nucleic acids are collectively known as "xeno-nucleic acids" or XNA, examples of which are shown in Figure 1.[5] Some of these XNA can even be replicated, reversed-transcribed, and evolved with engineered polymerases.[6]

While the concept of redesigning nucleic acids by completely replacing the sugar−phosphate backbone with other polymeric backbones is not entirely new (see Figure 2 for examples), decent Watson−Crick base pairing in these synthetic systems has been demonstrated only relatively recently. In 1991, Nielsen et al.[7] reported the first peptide nucleic acid (PNA) that could recognize double-stranded (ds) DNA through a novel triplex invasion mechanism. PNA also binds to single-stranded DNA/RNA with high affinity and specificity according to the Watson−

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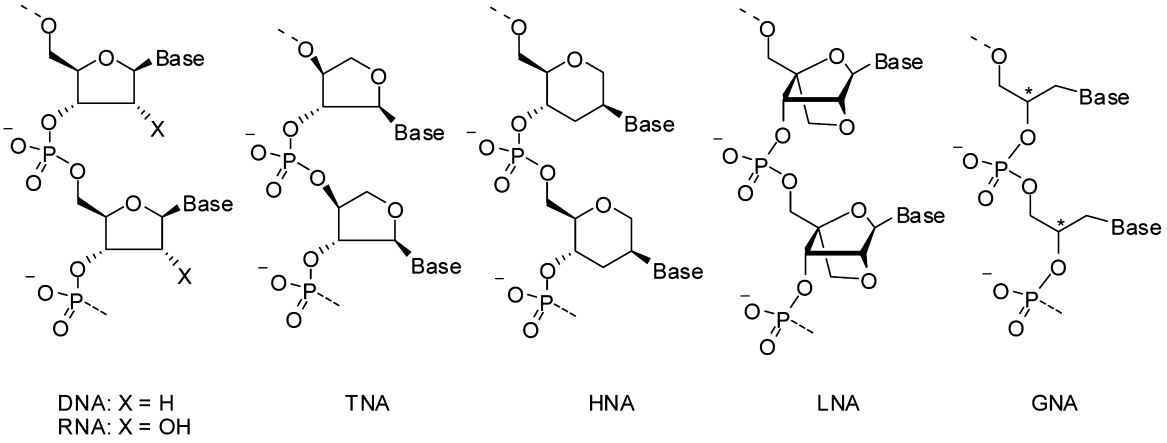

Figure 1. DNA/RNA and selected examples of xeno-nucleic acids.

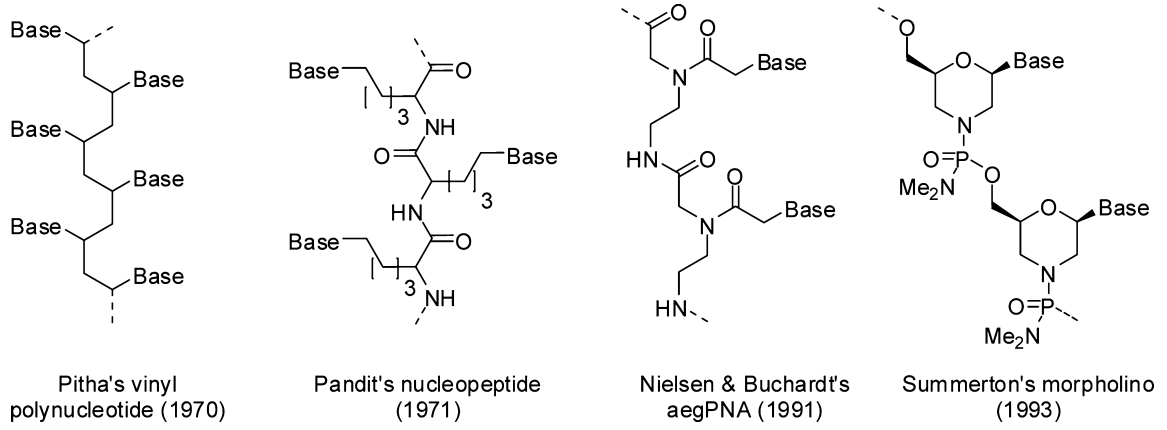

Figure 2. Examples of completely redesigned nucleic acid mimics.

Crick base-pairing rules. The electrostatically neutral backbone of PNA accounts for the unusually high stability as well as the relative insensitivity to ionic strength variations of PNA-containing duplexes.[8] These properties, together with the excellent stability of PNA toward nucleases and proteases, open the door to its use in nucleic-acid-related applications and considerably broaden the potential scope of this field.[9] In particular, the strong affinity of PNA is highly desirable for targeting of nucleic acids with secondary structures.[10] In addition, PNA is also interesting as a model for studying molecular recognition events, in prebiotic chemistry, and in designing self-replicating systems.[11,12] The virtually unlimited opportunities for structural modification should inspire the development of new PNAs having improved properties and functions.[13] In this Account, we summarize our contribution to the PNA research landscape, which focuses on the development and utility of a series of conformationally constrained pyrrolidinyl PNAs consisting of a proline-derived dipeptide backbone.

2. CONFORMATIONALLY CONSTRAINED PNA

The X-ray and NMR structures of PNA·PNA, PNA·RNA, and PNA·DNA duplexes suggest that the *N*-2-aminoethylglycine backbone of the original PNA (now known as aegPNA) is flexible enough to adopt a range of conformations, including the A-, B-, and novel P-type helices.[8] The concept of preorganizing the PNA structure to reduce the entropic penalty associated with the conformational change has been proposed to further enhance the binding properties (Figure 3).[14] Partial incorporation of the (*S*,*S*)-*trans*-cyclopentane ring into the aegPNA backbone improves its thermal stability and sequence specificity for DNA recognition.[15] On the other hand, incorporation of the *cis*-cyclohexane ring results in an increase in RNA binding affinity,

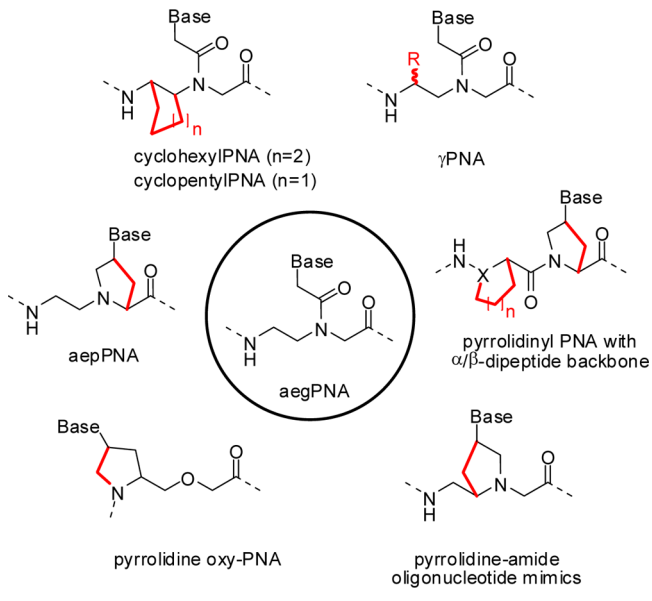

Figure 3. Examples of conformationally constrained PNA analogues.

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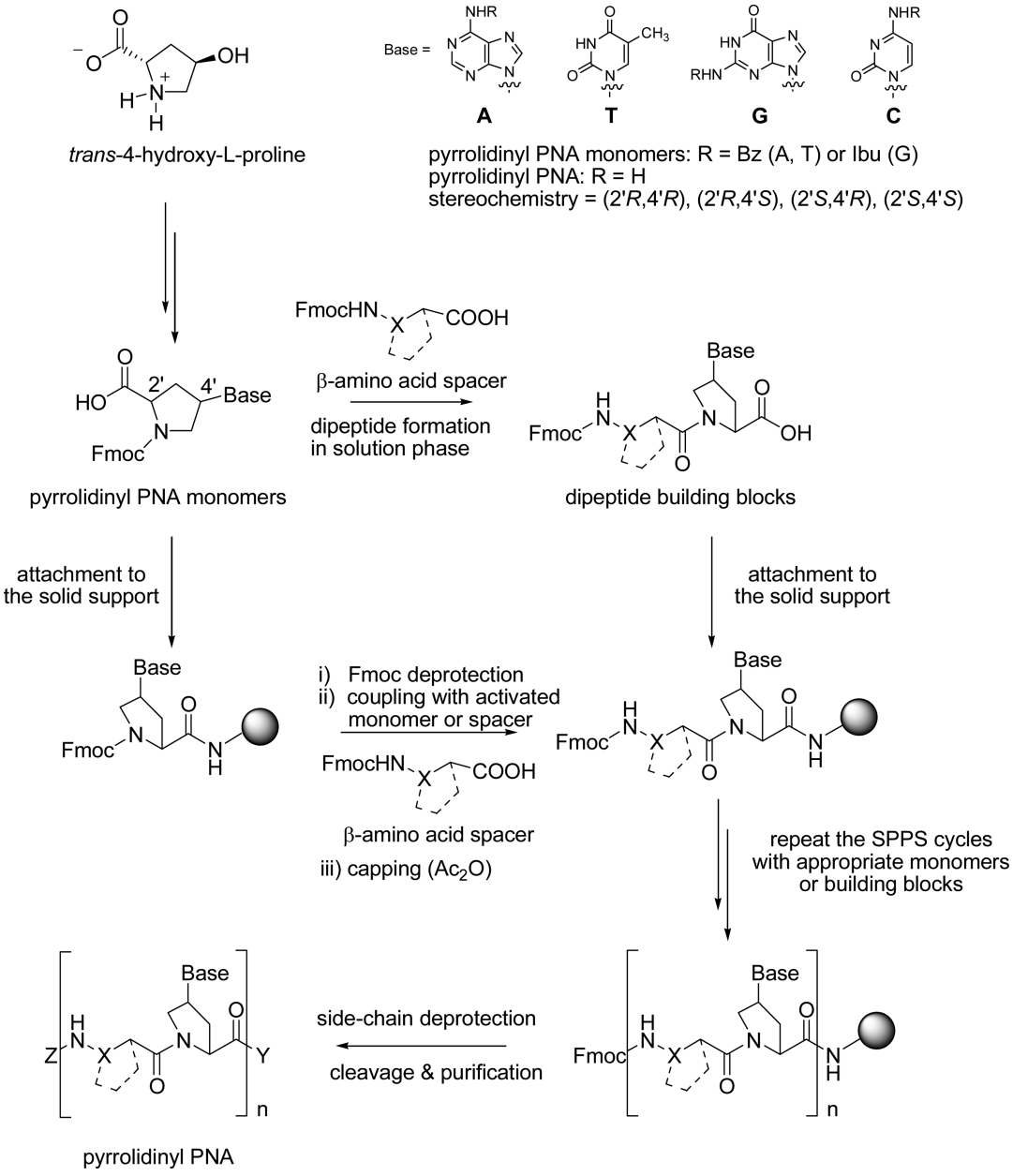

Figure 4. Synthesis of pyrrolidinyl PNA.

significantly more than for DNA.[16] Also, preorganization resulting from a simple substitution at the γ-position in aegPNA significantly improves its binding properties.[17] Various substituents can be placed at this position to improve water solubility[18] or cell penetration[19,20] or to incorporate other functions.[21,22]

Because of its straightforward access via proline derivatives having well-defined stereochemistries, the pyrrolidine ring has been extensively used as a constraint element in the design of new PNA structures (Figure 3). However, few studies have fully evaluated the general base-pairing behaviors of nonchimeric mixed-sequence pyrrolidine-containing PNA,[23,24] and none of these offer significant advantages over the well-established aegPNA. In 1996 we introduced a new pyrrolidinyl PNA having a modular dipeptide backbone consisting of nucleobase-modified prolines alternating with other amino acid "spacer" units.[25] However, it was not until 2001 that the active role of the cyclic β-amino acid spacer toward DNA binding was realized.[26] These findings were coincident with the discovery that short β-peptides can fold into well-defined helices as a result of intramolecular hydrogen bonding and conformation constraints.[27]

3. SYNTHESIS OF PYRROLIDINYL PNA

The structure of pyrrolidinyl PNA is divided into two parts: the pyrrolidine monomer and the β-amino acid spacer, which are equivalent to the nucleoside and phosphate groups in DNA, respectively. The four Fmoc-protected pyrrolidine monomers (A, T, C, G) were synthesized with full stereochemical control starting from *trans*-4-hydroxy-L-proline (Figure 4).[28−30] The nucleobase protecting groups Bz (A, C) and Ibu (G) chosen here are compatible with the Fmoc solid-phase peptide synthesis (SPPS) strategy and can be removed by aqueous ammonia treatment as in standard DNA synthesis. In early studies, the pyrrolidine monomer and the spacer were first joined to form a "nucleotide" building block before being assembled into PNA oligomers using SPPS.[31] The attractive feature of this approach is

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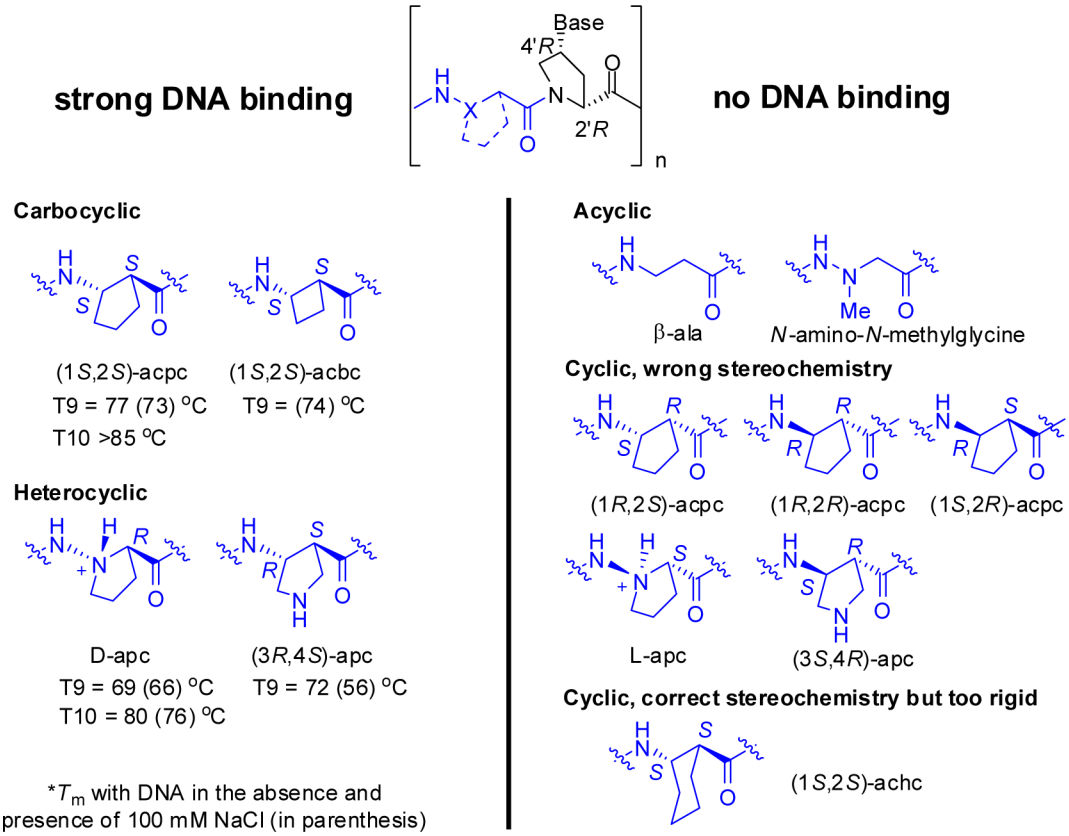

Figure 5. DNA binding properties of homothymine pyrrolidinyl PNAs having β-amino acid spacers.

that the number of solid-phase coupling steps is reduced by half. However, stepwise assembly of the monomer and spacer directly onto the solid phase is more convenient for building in structural variations, and this became the preferred method in later studies.[26,29] L-Lysinamide is usually included at either the C- or N-terminus to impart aqueous solubility (to the mM range). Other labels such as fluorescent dyes, thiols, and biotin may also be incorporated at either the N- or C-terminus (via an orthogonally protected lysine side chain). After the desired sequence is obtained, the nucleobase protecting groups are removed prior to cleavage from the solid support. While aegPNA and pyrrolidinyl PNA consisting of α-amino acids in the backbone are readily degraded by intramolecular cyclization under basic conditions,[25] pyrrolidinyl PNA having an α/β-dipeptide backbone is completely stable. The crude PNA is purified by reversed-phase HPLC and characterized by MALDI-TOF mass spectrometry. Typical coupling yields are >95% per step, and the recovery of PNA with >90% purity is between 10−40%, depending on the sequence.

4. BASE-PAIRING PROPERTIES OF PYRROLIDINYL PNA

4.1. DNA Binding Properties

During the discovery phase, the most easily prepared oligothymine sequences (5−10 bases in length) were used as a model to preliminarily determine the DNA binding properties of pyrrolidinyl PNA. The configuration of the pyrrolidine monomer was initially fixed as (2′*R*,4′*R*) to mimic that of natural nucleosides, and a limited set of β-amino acids were employed as spacers (Figure 5). A stable PNA·DNA hybrid (T_m = 80 °C for the T_{10} sequence) was first observed in dapcPNA having the (2*R*)-1-aminopyrrolidine-2-carboxylic acid (D-apc) spacer.[26,32] UV and circular dichroism (CD) titrations clearly revealed a 1:1 stoichiometry, which is in sharp contrast to other PNA systems, wherein triplex formation was preferred for homopyrimidine sequences.[8] The CD spectra of the PNA·DNA duplexes exhibit features that are compatible with right-handed helices.[32] As a hydrazino peptide, dapcPNA could potentially exist in its protonated form at physiological pH, which might provide additional attractive interactions with the negatively charged DNA backbone. The absence of DNA binding for pyrrolidinyl PNAs carrying either charged or uncharged open-chain spacers (β-alanine and *N*-amino-*N*-methylglycine) suggests the importance of structural constraints over electrostatic interactions to the DNA binding.[33] This hypothesis was further confirmed in subsequent studies of PNA with carbocyclic 2-aminocyclopentanecarboxylic acid (acpc) spacers. Out of the four possible diastereomers of acpc, only the pyrrolidinyl PNA having a (2′*R*,4′*R*)-pyrrolidine/(1*S*,2*S*)-acpc backbone (henceforth denoted as acpcPNA) formed a very stable hybrid with DNA (T_m > 85 °C for the T_{10} sequence).[30,34] The other two cyclic β-amino acids that promote DNA binding are (1*S*,2*S*)-2-aminocyclobutanecarboxylic acid [(1*S*,2*S*)-acbc][35] and (3*R*)-aminopyrrolidine-(4*S*)-carboxylic acid [(3*R*,4*S*)-apc)].[36] All of these have the same absolute stereochemistry as (1*S*,2*S*)-acpc and the protonated form of D-apc. Interestingly, although (1*S*,2*S*)-2-aminocyclohexanecarboxylic acid [(1*S*,2*S*)-achc] possesses the same configuration, achcPNA derived from this spacer cannot bind to DNA (vide infra). This underscores the importance of careful design and correct placements of conformational constraints in PNA systems.

After the key structural elements that are important for DNA binding had been established, more detailed studies directed to determining the DNA/RNA binding specificity and self-pairing

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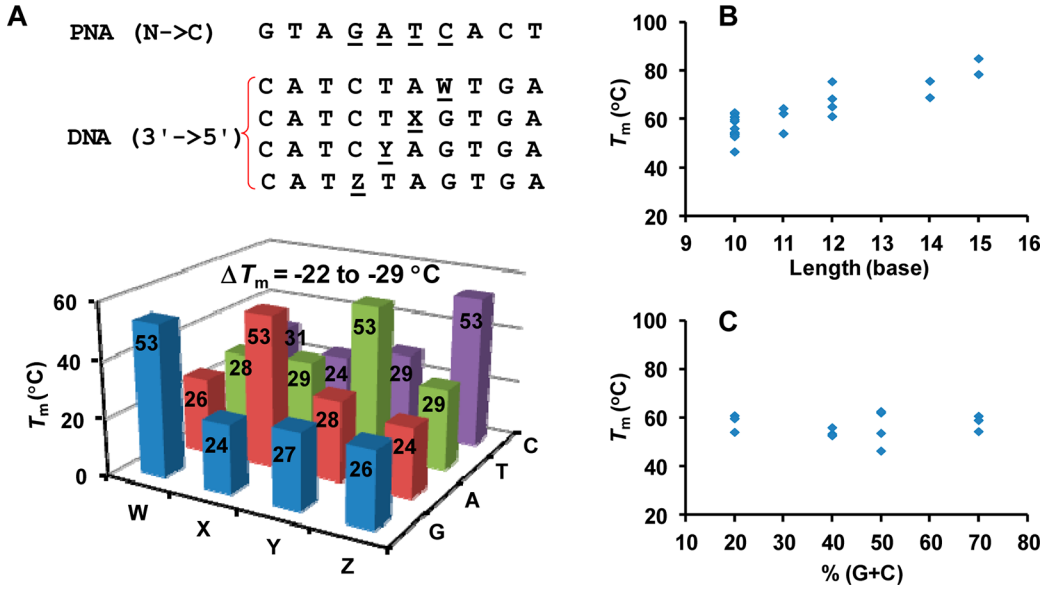

Figure 6. T_m of antiparallel acpcPNA·DNA duplexes. (A) Watson−Crick base-pairing specificity, (B) Relationships with number of base pairs (n = 24). (C) Relationships with base composition (10-mer, n = 13).

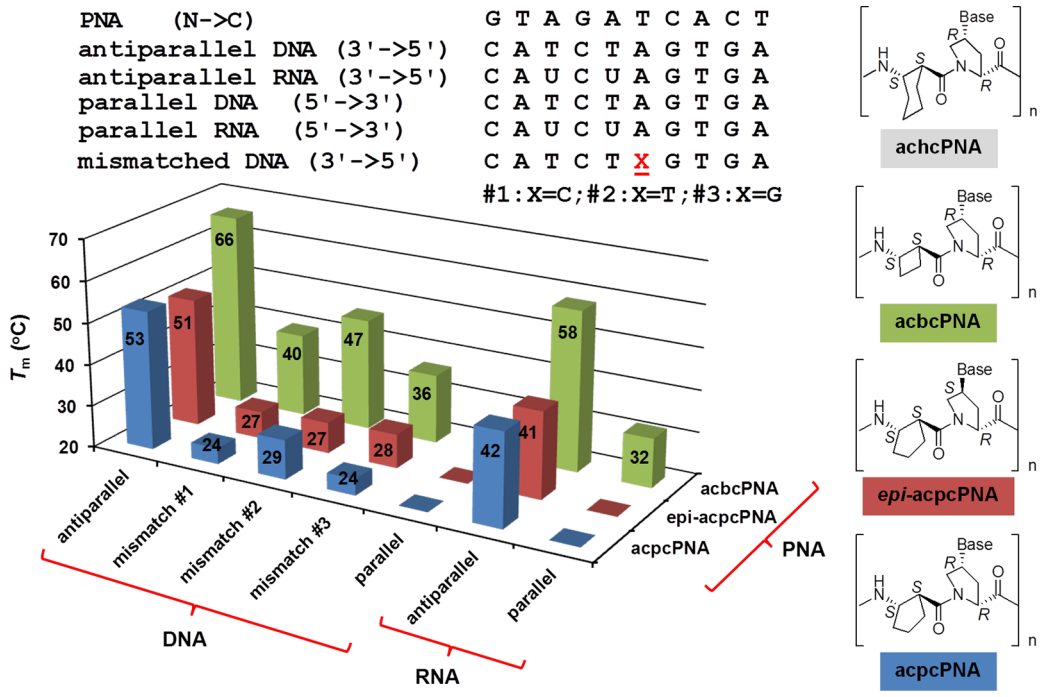

Figure 7. Comparison of T_m data of DNA and RNA hybrids of acpcPNA,[30] *epi*-acpcPNA,[38] and acbcPNA.[35] Values less than 20 °C are presented as 20 °C. achcPNA showed no detectable binding to either DNA and RNA (T_m < 20 °C in all cases).

ability were performed with selected PNA systems having mixed-base nonchimeric sequences. While aegPNA is known to form stable hybrids in both the antiparallel and parallel directions, acpcPNA exclusively forms antiparallel hybrids with DNA.[29] The pairing between acpcPNA and DNA exhibits excellent Watson−Crick specificity, as shown by an extremely large T_m decrease (22−29 °C) in the mismatched hybrids compared with the complementary hybrids (Figure 6A).[30] The T_m of acpcPNA·DNA hybrids increases with the number of base pairs (Figure 6B) but does not show obvious correlations with the base composition (G + C and purine/pyrimidine content) (Figure 6C). Whether this is a general phenomenon for the pyrrolidinyl PNA family remains to be confirmed, but preliminary studies with *epi*-acpcPNA and acbcPNA appear to suggest otherwise. An analysis of thermodynamic parameters suggests that the larger enthalpy gain due to G·C pairing relative to A·T pairing is counterbalanced by the more negative entropy term in the former case.[30] The different extents of puckering of the proline ring due to differences in electronegativity and steric bulkiness of the nucleobase substituents may partly explain such unusual sequence-dependent effects.[37]

The effect of the pyrrolidine stereochemistry was next explored by employing (1*S*,2*S*)-acpc as the spacer.[30] Out of three remaining diastereomeric acpcPNAs, only the diaster-

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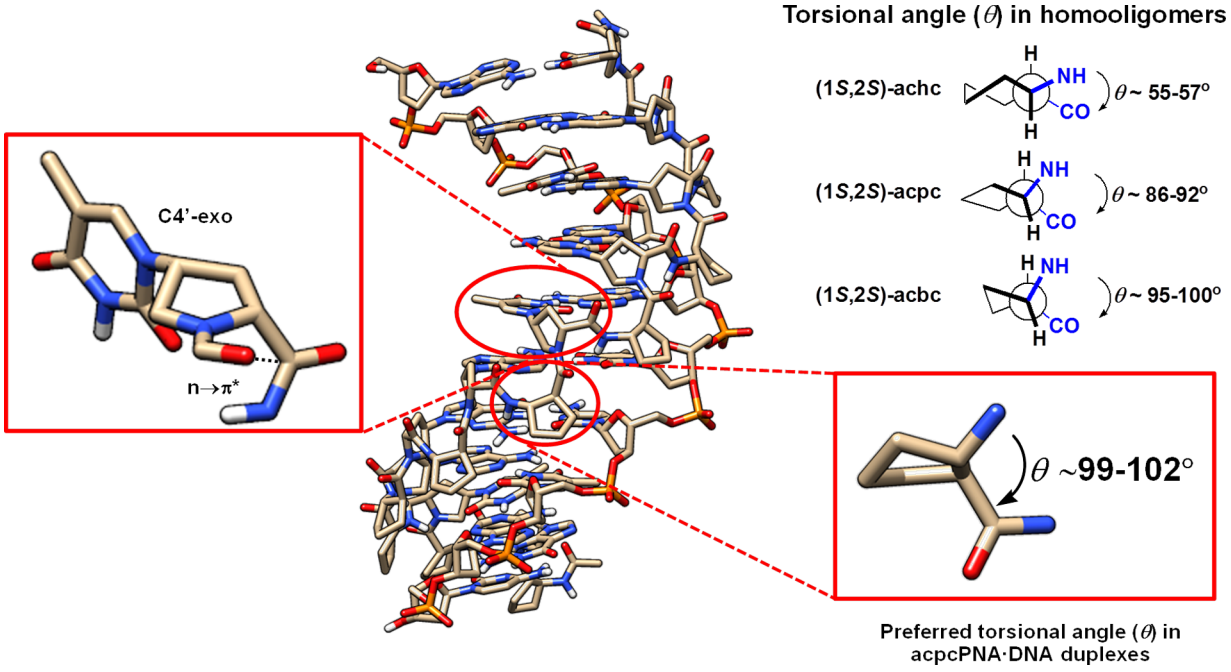

Figure 8. Model of the acpcPNA·DNA duplex showing the conformation of the pyrrolidine ring and torsional angles of the cyclic β-amino acids. Adapted from ref 35. Copyright 2012 American Chemical Society.

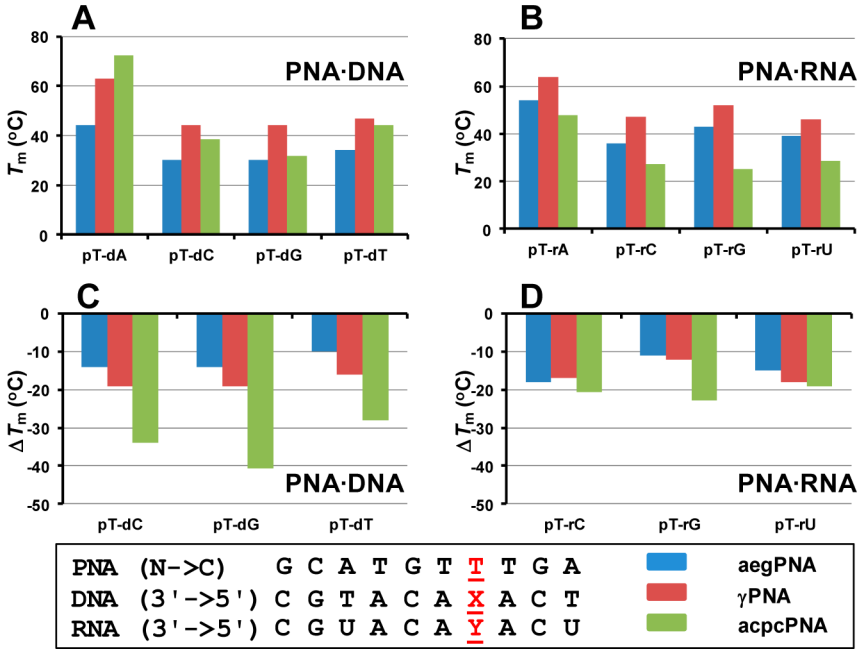

Figure 9. T_m and ΔT_m ($=T_m^{\text{mismatched}} - T_m^{\text{complementary}}$) data for (A, C) DNA hybrids and (B, D) RNA hybrids of aegPNA, γPNA, and acpcPNA under comparable conditions. The L-serine-derived γPNA was fully modified with hydroxymethyl side chains.[17]

eomer with the (2′*R*,4′*S*)-pyrrolidine/(1*S*,2*S*)-acpc backbone (henceforth denoted as *epi*-acpcPNA) shows appreciable DNA binding affinity, which is only slightly lower than that of the acpcPNA having the (2′*R*,4′*R*)-pyrrolidine/(1*S*,2*S*)-acpc backbone (Figure 7).[30,38] This behavior is analogous to that of α-anomeric DNA, which can form Watson−Crick pairs with normal (β) DNA.[39]

Importantly, decreasing the ring size of the cyclic β-amino acid spacer from five carbons in acpcPNA to four carbons in acbcPNA resulted in more stable PNA·DNA duplexes without compromising the base-pairing specificity, while achcPNA having a six-membered-ring spacer showed no ability to bind DNA. This can be rationalized by considering the NH−C2−C1−CO torsional angle (θ) in the cyclic β-amino acid (Figure 8).[35] The native θ value of 95−100°, obtained from an X-ray structure of oligo(*trans*-acbc),[40] is closer to the values of 99−102° obtained from molecular dynamics (MD) simulations of dapcPNA·DNA[41] and acpcPNA·DNA[42] duplexes than those of *trans*-acpc (86−92°), and *trans*-achc (55−57°).[43] The rigid six-membered ring in *trans*-achc is likely to be far less flexible to adopt the desired optimal torsional angle for the DNA binding conformation compared with the five-membered ring in *trans*-

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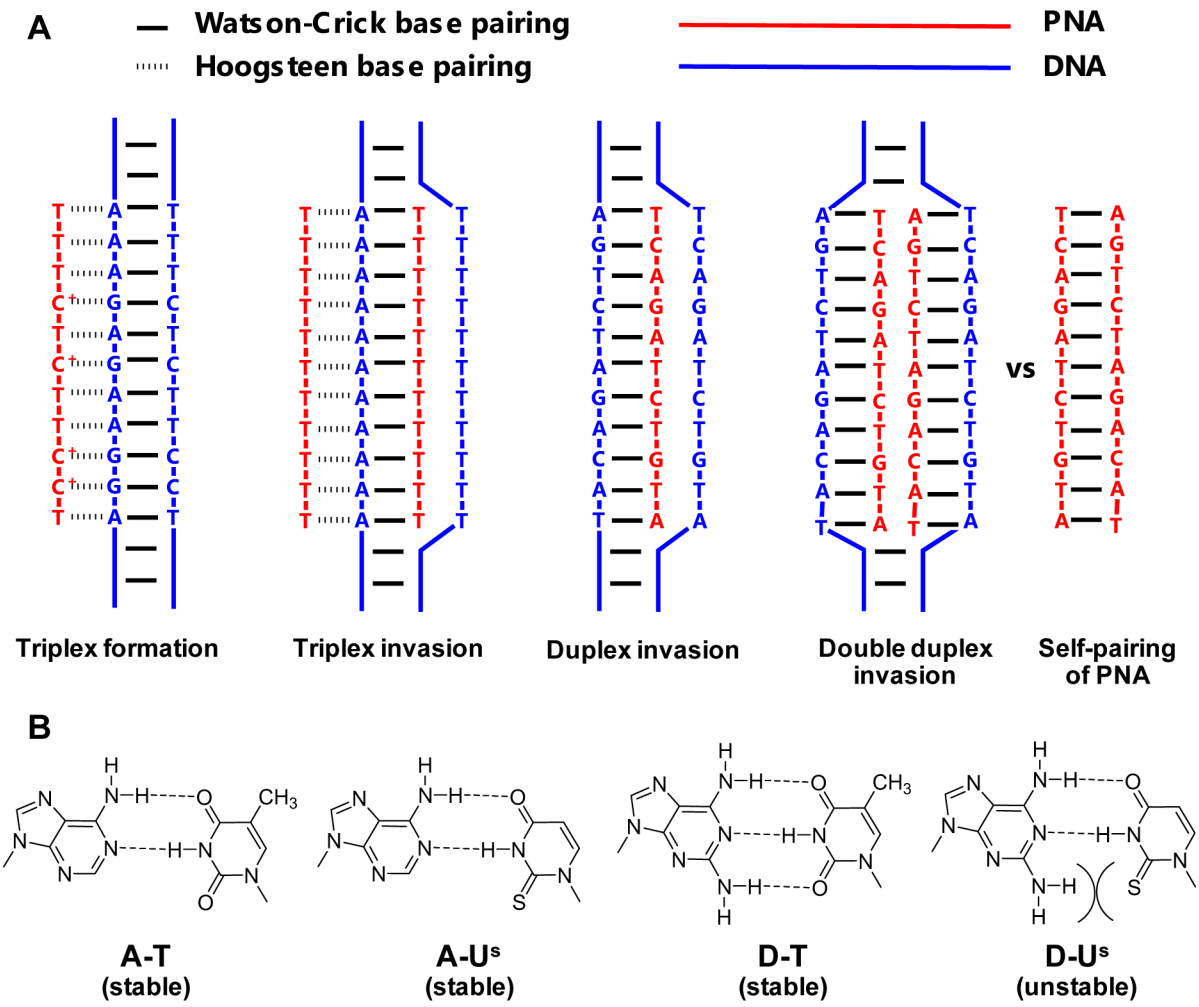

Figure 10. (A) Various modes of dsDNA recognition by PNA and (B) the concept of "pseudocomplementary" bases. Adapted with permission from ref 46. Copyright 1999 The National Academy of Sciences.

acpc. The MD simulations suggest that all of the amide bonds adopt a trans geometry, allowing n → π^* interactions between adjacent amide bonds in the N → C direction. In addition, they also suggest that the gas-phase enthalpy, calculated from a summation of bonded and nonbonded terms, is the major contributor to the high stability of pyrrolidinyl PNA·DNA duplexes, which is consistent with the uncharged nature of the PNA backbone. This is in contrast to DNA·DNA duplexes, in which the gas-phase energy is high because of electrostatic repulsion, and thus solvation becomes the predominant stabilizing factor. A comparative simulation study of acpcPNA·DNA and *epi*-acpcPNA·DNA duplexes suggests that both the (2′*R*,4′*R*) and (2′*R*,4′*S*) pyrrolidine moieties can be well-accommodated in antiparallel B-DNA-like duplexes by adopting different ring-puckering modes.[42] The (2′*R*,4′*R*) acpcPNA showed C4′-exo puckering of the pyrrolidine ring while the (2′*R*,4′*S*) *epi*-acpcPNA showed C4′-endo puckering, placing the nucleobases in pseudoequatorial positions in both cases.

The availability of literature data for aegPNA and γPNA[17−19] allows a direct comparison of their DNA and RNA binding properties with those of acpcPNA (Figure 9). The T_m of the complementary acpcPNA·DNA hybrid (72 °C) was higher than those of aegPNA and γPNA (44 and 63 °C, respectively). Moreover, mismatched DNA hybrids of acpcPNA showed larger T_m decreases (28−41 °C) than in aegPNA (10−14 °C), and γPNA (16−19 °C). The superior mismatch discrimination abilities of acpcPNA over aegPNA and DNA probes has also been clearly demonstrated by surface plasmon resonance (SPR)[44] and magnetic bead DNA capture experiments.[45]

4.2. RNA Binding and Self-Pairing Properties

While most other PNAs bind more strongly to RNA over DNA, pyrrolidinyl PNAs consistently show preferential DNA binding. The antiparallel binding mode is clearly preferred in pyrrolidinyl PNA·RNA hybrids, although weak parallel hybrids are also occasionally observed.[30] The data in Figures 7 and 9 indicate that acpcPNA forms less stable RNA hybrids than aegPNA or γPNA. Nevertheless, the T_m of 48 °C at 10 base pairs is still much higher than that of natural DNA/RNA pairs. Moreover, the larger T_m decrement for mismatched acpcPNA·RNA hybrids (19−23 °C) suggests a greater specificity compared with aegPNA (11−15 °C) and γPNA (12−18 °C). Although the structural basis of the much higher thermal stability of acbcPNA·RNA hybrids relative to acpcPNA·RNA hybrids is not yet understood,[35] acbcPNA is clearly more useful for RNA-related applications.

Perhaps the most striking feature of pyrrolidinyl PNA is the low propensity for self-pairing. While *epi*-acpcPNA forms only weakly stable self-hybrids,[38] no self-pairing has been observed in acpcPNA.[30] The order of pyrrolidinyl PNA duplex stabilities (PNA·DNA > PNA·RNA > PNA·PNA) is therefore completely opposite to that of aegPNA (PNA·PNA > PNA·RNA > PNA·

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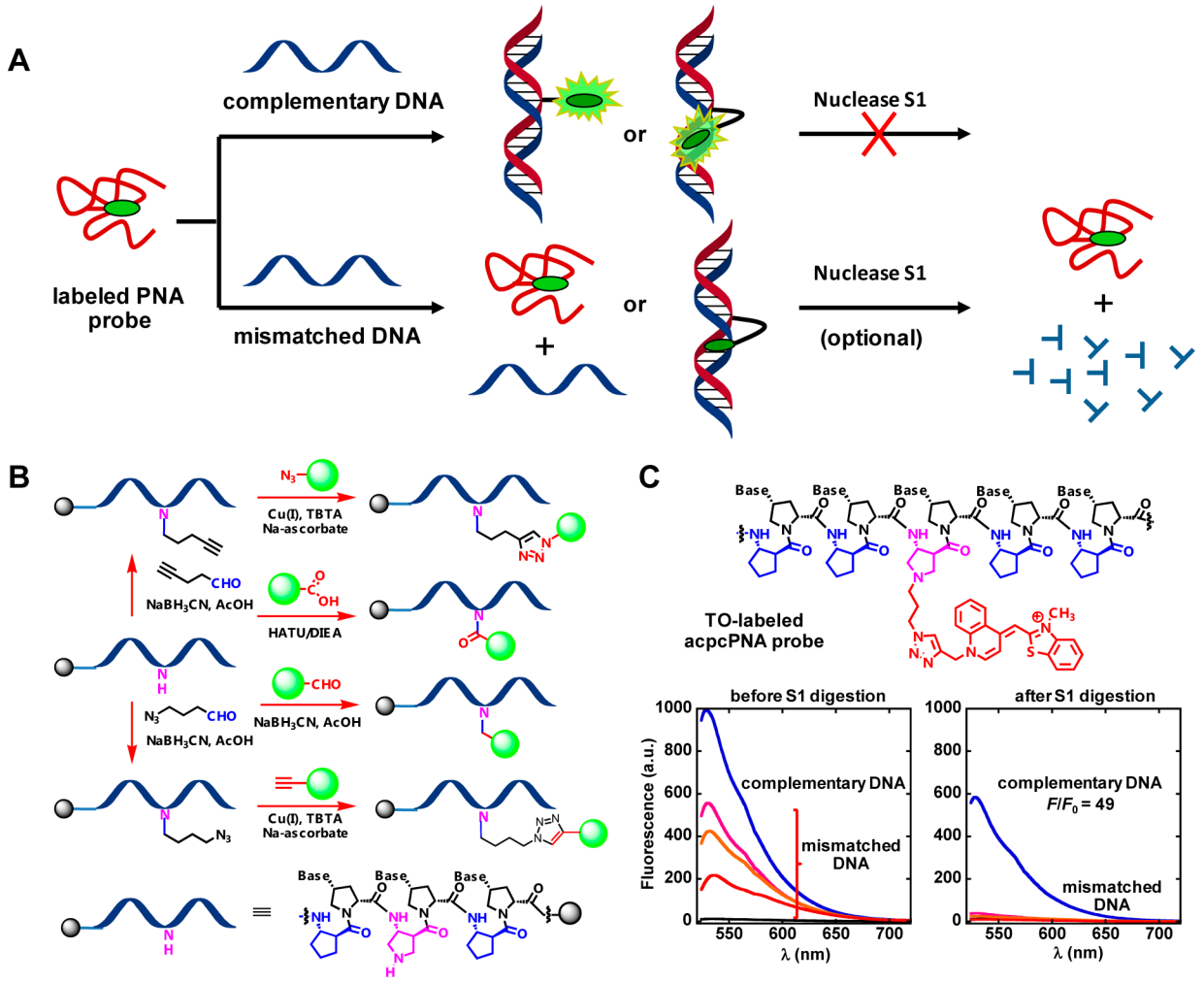

Figure 11. (A) Working principle of self-reporting PNA probes. (B) Strategies for backbone labeling of acpcPNA. Adapted from ref 58. Copyright 2013 American Chemical Society. (C) DNA-hybridization-responsive acpcPNA probes.

DNA). Destabilization of the acpcPNA self-hybrids was believed to be a consequence of the steric bulk of the acpcPNA strands. This hypothesis was experimentally supported by the observation that the less bulky acbcPNA formed considerably more stable self-hybrids than acpcPNA.[35] However, the greater stability of self-hybrids of *epi*-acpcPNA over acpcPNA also suggests the importance of stereochemistry to the self-pairing ability. NMR studies of three-dimensional structures as well as theoretical calculations are currently underway with the aim of further clarifying this issue. The obvious consequence of non-self-pairing PNA is that there is more flexibility in terms of sequence design without the concern of self-complementarity. On the other hand, such PNAs may not be suitable for applications that require self-pairing, such as in the construction of purely PNA-based nanomaterials, but they should still be useful when being used in combination with DNA such as in the development of DNA origami.[3,4]

PNA is among the few synthetic molecules that can recognize structured nucleic acid targets, including dsDNA, in a sequence-specific fashion.[10] In principle, double duplex invasion by PNA allows targeting of dsDNA without being limited to only purine rich regions as in triplex formation or triplex invasion (Figure 10A). However, the extremely high stability of aegPNA·aegPNA duplexes disfavors the double duplex invasion by two complementary strands of aegPNA designed to target the same region of dsDNA. Replacement of the base A with diaminopurine (D) and the base T with 2-thiouracil (U^s) gave "pseudocomplementary" aegPNA having improved DNA invasion ability through destabilization of aegPNA·aegPNA duplexes and stabilization of aegPNA·DNA duplexes (Figure 10B).[46] Nevertheless, such pseudocomplementary base modification exists only for A·T pairs, and therefore, this may not completely suppress self-pairing in G·C-rich aegPNA sequences. In such cases, a combination of base modification and other strategies such as incorporation of positive charges into the aegPNA backbone is necessary.[47] Since acpcPNA is inherently pseudocomplementary, it should be a potential candidate for double duplex invasion of dsDNA without concerns over sequence-related limitations. Although a recent finding showed that unmodified γPNA with sufficient length can directly invade into dsDNA without the need for the complementary PNA strand,[48] invasion by two strands of acpcPNA simultaneously should provide a much larger driving force. It also suggests the possibility of using single-stranded pyrrolidinyl PNA to directly invade into dsDNA in a similar fashion.

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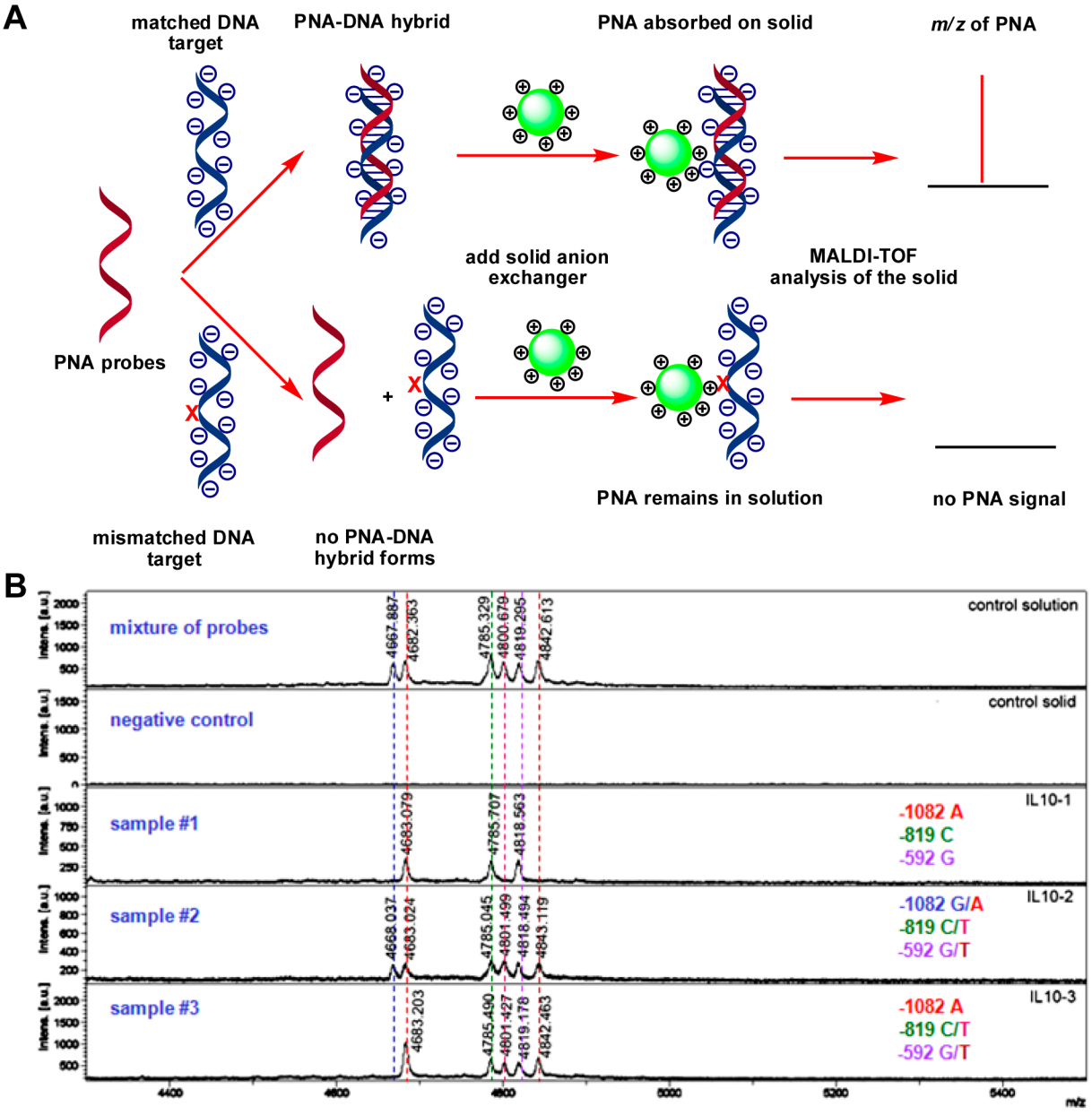

Figure 12. (A) Electrostatic capture of PNA·DNA hybrids. (B) SNP typing of human *IL-10* promotor region by MALDI-TOF analyses of the captured acpcPNA probes. Adapted from ref 63. Copyright 2008 American Chemical Society.

5. APPLICATIONS OF PYRROLIDINYL PNA

5.1. Sensor Probes

The excellent chemical and biological stability, the ability to form stable hybrids at low ionic strength, and the ability to recognize structured nucleic acid targets by strand invasion or triplex formation suggest the promising potential of PNA to improve the performance of nucleic acid biosensors.[49] Excellent mismatch discrimination has been demonstrated in various acpcPNA-based DNA detection platforms, including quartz crystal microbalance (QCM),[50] SPR,[44,51] voltammetric,[52] and capacitive detection.[53,54]

5.2. Self-Reporting Fluorescence Probes

PNA generally adopts a compact structure in aqueous solution, thereby ensuring that dye-labeled single-stranded PNAs have distinctly different environments from duplexes without the requirement of a stem−loop structure as in classical beacons.[55] This, together with the potential for better mismatch discrimination, prompted us to develop self-reporting fluorescence acpcPNA probes (Figure 11A). The ability of PNA to protect DNA from digestion by S1 nuclease offers a unique opportunity to improve the discrimination further.[56]

As an example, 5-pyren-1-yluracil (U^{Py}) incorporated into acpcPNA can specifically recognize dA in DNA and provides a strong fluorescence increase at 465 nm upon base pairing (3−42-fold, depending on the sequence).[57] This fluorescence enhancement was interpreted in terms of changes in the local environment and twisting between the pyrene and uracil moieties, which controls the degree of charge transfer.

To facilitate backbone labeling in acpcPNA, (3*R*,4*S*)-3-aminopyrrolidine-4-carboxylic acid (apc) was incorporated into the acpcPNA backbone to create a chimeric apc/acpcPNA. The (3*R*,4*S*)-apc spacer is structurally compatible with the acpcPNA backbone, as shown by the observation of very little

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destabilization of the apc-containing PNA·DNA duplexes.[36] The inserted apc spacer provides a convenient handle for the introduction of various dyes or labels to the acpcPNA backbone via acylation, reductive alkylation, or click chemistry in a postsynthetic fashion (Figure 11B).[58] Pyrene appended to the acpcPNA backbone through a flexible linker is efficiently quenched by adjacent nucleobases ($T > C > G \gg A$) in the single-stranded acpcPNA. Hybridization with the DNA target restores the fluorescence (2.9−73-fold fluorescence increase). MD simulations suggest that the pyrene is located in the minor groove of the acpcPNA·DNA duplex and thus can no longer interact with the nucleobases.[59]

The unsymmetrical cyanine dye thiazole orange (TO) has been extensively used in combination with aegPNA in light-up[60] and forced intercalation (FIT) probes.[61] acpcPNA with a TO-labeled backbone shows an extremely large fluorescence increase upon hybridization to DNA. Nonspecific binding can be eliminated by S1 nuclease digestion, resulting in excellent mismatch discrimination (Figure 11C).[58] The solvatochromic benzophenoxazine dye Nile red attached to the acpcPNA backbone shows enhanced and blue-shifted fluorescence upon hybridization with DNA. This suggests that the Nile red in the PNA·DNA duplex is located in a more hydrophobic environment than in single-stranded PNA, and the effect may be used to probe local structures of the duplex such as bulge formation.[62]

5.3. DNA Sensing Based on Differential Electrostatic Properties of PNA and DNA

The electrostatically neutral backbone of PNA provides a unique means for the development of novel DNA sensing methods. The different electrostatic properties of acpcPNA and acpcPNA·DNA hybrids allows selective capture of the hybridized PNA probe by a positively charged solid support such as Q-sepharose[63] or polymer-coated magnetite particles.[64] MALDI-TOF mass spectrometry can be used for sensitive detection of the captured PNA probe and hence identification of the DNA sequence in a label-free fashion (Figure 12). The high specificity of acpcPNA allows simultaneous multiplex discrimination of single nucleotide polymorphism (SNP), which can be difficult to achieve with conventional PNA probes.[65]

A similar concept has been applied to the fabrication of a paper-based DNA sensor by functionalization of the cellulose paper with a positively charged polymer brush that can electrostatically capture the DNA sample together with its complementary biotinylated PNA probe. The presence or absence of captured PNA probes, which determines the identity of the DNA sequence, is revealed by an enzymatically amplified colorimetric reaction.[66]

6. CONCLUSION AND OUTLOOK

This Account has highlighted our contribution to the development of conformationally constrained pyrrolidinyl PNAs having α/β-dipeptide backbones. The modular nature of the design enables the rapid synthesis and exploration of the base-pairing properties of these systems. AcpcPNA, a representative member of such pyrrolidinyl PNA systems, binds to DNA with excellent affinity and specificity. In addition, acpcPNA possesses other unique and potentially useful characteristics: strong antiparallel selectivity, the preference for pairing to DNA over RNA, and the unprecedented inability to form self-hybrids. It is important to note that only minor changes in the pyrrolidinyl PNA structure can lead to significantly different base-pairing behaviors, allowing one to fine-tune properties such as self-pairing or RNA binding to suit the desired application. Although the structural contribution of the amino acid part to the binding characteristics of pyrrolidinyl PNA is largely understood, little is known about the effect of the pyrrolidine part, and there are opportunities for improvement. Understanding factors contributing to these unusual behaviors through a combination of structural and theoretical studies will contribute to targeted, tailored "new generation" pyrrolidinyl PNAs with improved/customizable properties. Finally, while diagnostic applications of pyrrolidinyl PNA have been clearly demonstrated, its therapeutic/biological applications[67] and other areas such as functional materials[68] are only emerging. We invite others to join us as we continue to explore the full potential of utilizing acpcPNA and related pyrrolidinyl PNAs in these cutting-edge research areas and beyond.

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