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Gamma Peptide Nucleic Acids: As Orthogonal Nucleic Acid Recognition Codes for Organizing Molecular Self-Assembly

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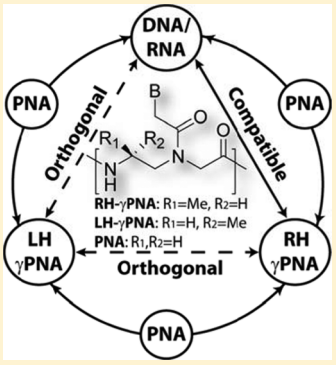
Supporting Information

ABSTRACT: Nucleic acids are an attractive platform for organizing molecular self-assembly because of their specific nucleobase interactions and defined length scale. Routinely employed in the organization and assembly of materials *in vitro*, however, they have rarely been exploited *in vivo*, due to the concerns for enzymatic degradation and cross-hybridization with the host's genetic materials. Herein we report the development of a tight-binding, orthogonal, synthetically versatile, and informationally interfaced nucleic acid platform for programming molecular interactions, with implications for *in vivo* molecular assembly and computing. The system consists of three molecular entities: the right-handed and left-handed conformers and a nonhelical domain. The first two are orthogonal to each other in recognition, while the third is capable of binding to both, providing a means for interfacing the two conformers as well as the natural nucleic acid biopolymers (i.e., DNA and RNA). The three molecular entities are prepared from the same monomeric chemical scaffold, with the exception of the stereochemistry or lack thereof at the γ-backbone that determines if the corresponding oligo adopts a right-handed or left-handed helix, or a nonhelical motif. These conformers hybridize to each other with exquisite affinity, sequence selectivity, and level of orthogonality. Recognition modules as short as five nucleotides in length are capable of organizing molecular assembly.



INTRODUCTION

Nucleic acids, particularly DNA oligonucleotides, have emerged over the past three decades as valuable tools for organizing molecular self-assembly because of their specific and predictable nucleobase interactions and defined length scale.[1] The molecular architecture of a given system, in principle, could be determined *a priori* by encoding each building block with a specific recognition code whose interaction is defined by the A-T (or A-U) and C-G base pairing and length scale defined by the 3.4 Å distance between each base-pair rung, in a manner similar to the production of LEGO blocks with pegs and holes for construction of toy models.[2] This programmable, molecular self-assembly approach has been exploited in the construction of a large variety of macro- and supramolecular systems[3] including proteins,[4] synthetic polymers,[5] dendrimers,[6] nanoparticles,[7] DNA tiles and origami,[8−14] as well as a slew of dynamic ensembles[15] including molecular computing,[16,17] logic circuits,[18−22] nanomachines and devices,[23−29] template-directed synthesis,[30] and hybridization chain reaction (HCR).[31] However, despite its broad utility *in vitro*, such a concept has rarely been exploited in the organization and assembly of materials *in vivo*,[32] due largely to the enzymatic lability and lack of recognition orthogonality of the natural nucleic acid "velcros."

The primary concerns for utilization of natural nucleic acids as molecular assembly and computing elements *in vivo* are nucleolytic degradation and cross-hybridization with background genetic materials. Such occurrences would be counterproductive and could lead to premature disassembly of the complex, degradation of molecular signals, and induction of cytotoxicity. The rather weak base-pairing interactions of DNA or RNA also warrant the application of relatively long recognition modules, typically in the range of 15−30 nucleotides (nts) in length, in order to achieve the desired thermodynamic stability. Such a requirement places restriction on the length scale and level of compaction that could be built in a given system. A tighter binding synthetic analogue, such as a locked nucleic acid (LNA),[33] could be employed to enable the usage of shorter recognition elements;[33] however, such a nucleic acid mimic is relatively difficult to prepare and chemically modify, and costly. Moreover, it still lacks the recognition orthogonality that is necessary for *in vivo* molecular assembly, despite the development of the α-isomer (inversion of chirality at C2′, C3′, and C4′).[34] In that respect, unnatural α-DNA[35] or α-RNA (or "Spiegelmer")[36] could be employed since neither can effectively recognize the natural β-counterparts; however, recognition is not entirely orthogonal. Cross hybridization still occurs, but to a lesser extent. Even if such a truly orthogonal recognition system could be developed, presently there is not a simple way to interface the two enantiomeric modalities. Development of a nucleic acid system with both recognition orthogonality information-interfaced capability will provide greater ease and flexibility in the design and execution of molecular self-assembly and computing in a living system. Herein we report the

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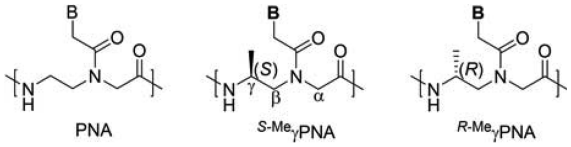
development of a tight-binding, orthogonal, synthetically versatile, and information-interfaced nucleic acid platform, called a gamma-peptide nucleic acid (γPNA), for programming molecular interactions. The system comprises three molecular entities: the right-handed (RH) and left-handed (LH) helical conformers and a nonhelical (NH) domain, with the first two incapable of recognizing each other, and the third capable of recognizing the RH and LH conformers, as well as the natural nucleic acid biopolymers (i.e., DNA and RNA), enabling the storage and translation of genetic information from one system to the next. All three domains are prepared from the same chemical building blocks, with the exception of the stereochemistry or lack thereof at the γ-backbone that determines if the corresponding oligo adopts an RH, LH, or NH motif (Chart 1). The work has direct implications for *in vitro* as well as *in vivo* molecular self-assembly and computing.

Chart 1. Chemical Structures of PNA and γPNA Units



RESULTS AND DISCUSSION

Rationale. Peptide nucleic acid (PNA) has been around for more than two decades, developed by Nielsen and co-workers[37] in 1991 in which the natural sugar phosphodiester backbone was replaced by achiral *N*-(2-aminoethyl)glycine units (Chart 1). PNA has many appealing features, including high binding affinity and sequence specificity, resistance to enzymatic degradation by proteases and nucleases, and an ability to invade certain sequences of double helical DNA.[38] However, because of the nonionic backbone, PNA is only moderately soluble in water and, as such, it has a tendency to aggregate and adhere to surfaces and other macromolecules in a nonspecific manner.[39,40] Such a property makes sample handling and processing less of a routine. Further, like most nucleic acids, PNA is not cell-permeable.[41] Such a barrier has prevented PNA from finding widespread applications in biology and medicine, though some progress has been made.[42−46] In the attempts to address these issues, diverse chemical modifications have been made to the structure of PNA.[47−49] Among the most promising modification—with respect to the ease of chemical synthesis, functional group diversification, and conformational preorganization—was the installation of a chiral center at the γ-backbone.[45,50−60] We showed that the enhancements in binding affinity and sequence selectivity of PNA upon introduction of an (*S*)-chiral center (prepared from an L-amino acid) at this position emanate from backbone preorganization, a conformational transition from a globular fold into an RH helical motif.[61,62] However, on the basis of these initial studies with limited stereochemical exploration, it was not clear whether the helical sense of γPNA could be reversed simply by switching the chirality at the γ-backbone and, if so, whether the resulting RH and LH conformers would be able to recognize each other, or how they would interact with DNA or RNA. To address these and other related questions, we synthesized the (*S*)- and (*R*)-γ-Me chiral PNA building blocks (Chart S1, Supporting Information) along with the corresponding oligos and characterized their conformations and hybridization properties and molecular self-assembly capability. We selected a pentameric sequence (Table 1), devoid of a self-hybridization possibility, as a starting point because our previous work showed that this particular class of chiral PNA exhibits unusually strong binding affinity and sequence selectivity for DNA as well as RNA.[63] Since PNA−PNA is generally thermodynamically more stable than a PNA−DNA or PNA−RNA duplex,[64] we surmised that a 5-mer sequence might be sufficient to form a stable duplex with its complementary partner.

Table 1. Sequence of PNA and γPNA Oligos[a]

PNA	Sequence	γ-Config.	Hel. sense
1	H-^{L}Lys-CCAAC-^{L}Lys-NH$_2$	Achiral	NH
2	H-^{L}Lys-GTTGG-^{L}Lys-NH$_2$	Achiral	NH
3	H-^{L}Lys-**CCAAC**-^{L}Lys-NH$_2$	S (L)	RH
4	H-^{L}Lys-**GTTGG**-^{L}Lys-NH$_2$	S (L)	RH
5	H-^{L}Lys-**CCAAC**-^{L}Lys-NH$_2$	R (D)	LH
6	H-^{L}Lys-**GTTGG**-^{L}Lys-NH$_2$	R (D)	LH
7	H-^{L}Lys-**CCGAC**-^{L}Lys-NH$_2$	R (D)	LH
8	H-^{L}Lys-**CCCAC**-^{L}Lys-NH$_2$	R (D)	LH
9	H-^{L}Lys-**CCTAC**-^{L}Lys-NH$_2$	R (D)	LH

[a]Bold indicates chiral γ-Me PNA unit. L and D indicate the stereochemical configurations of the amino acid (alanine) from which these chemical building blocks were prepared. NH: nonhelical, RH: right-hand, LH: left-hand.

Conformational Analysis. To determine the effect of γ-backbone chirality on the conformation of PNA, we measured the CD spectra of PNA1 through 6, individually and after hybridization to their partner strands containing matching sequence and helical sense. Note that PNA3 and 4 contained chemical building blocks with (*S*)-Me, and PNA5 through 9 with (*R*)-Me at the γ-backbone. We expected the first set to adopt an RH helical motif, as demonstrated in our earlier study,[61,63] while the helical sense of the latter set was unknown and needed to be determined. Previously we have shown that the stereochemistry of the terminal lysine residue has no bearing on the conformation or helical sense of PNA as an individual strand (Figure S1). Lysine residues were incorporated at the terminal positions of PNA to improve water solubility and minimize self-aggregation. The stereochemistry, L or D, was appropriately chosen to provide enantiomeric backbone uniformity with respect to the γ-position.

As expected, no CD signals were observed for PNA1 or 2 in the nucleobase absorption regions (200−320 nm), indicating that in the unhybridized (single-stranded) state PNA does not have a defined helical conformation (Figure 1A). We ruled out the possibility of PNA adopting a racemic mixture of RH and LH helices based on prior analysis.[61] However, in the case of PNA3 through 6, pronounced CD signals were observed. The CD spectrum of PNA3, with (*S*)-Me at the γ-backbone, exhibited a distinct exciton coupling pattern, with maxima at 268 and 221 nm, the minimum at 240 nm, and a crossover at 255 nm, characteristic of a right-handed helix.[64] We ruled out the possibility of self-hybridization based on concentration and temperature dependent CD measurements.[61] In contrast, PNA5, which contained an identical nucleobase sequence as that of PNA3 but with the opposite γ-backbone stereochemistry, displayed a mirror-image CD profile, in both pattern and amplitude, indicating that it formed an exact LH helical motif. Similar CD profiles were observed for PNA4 and 6, but they were slightly red-shifted and significantly weaker in signals compared to that of PNA3 and 5 (Figure 1A, *Inset*), despite the fact that they were of the same concentrations (Figures S2 and S3). One

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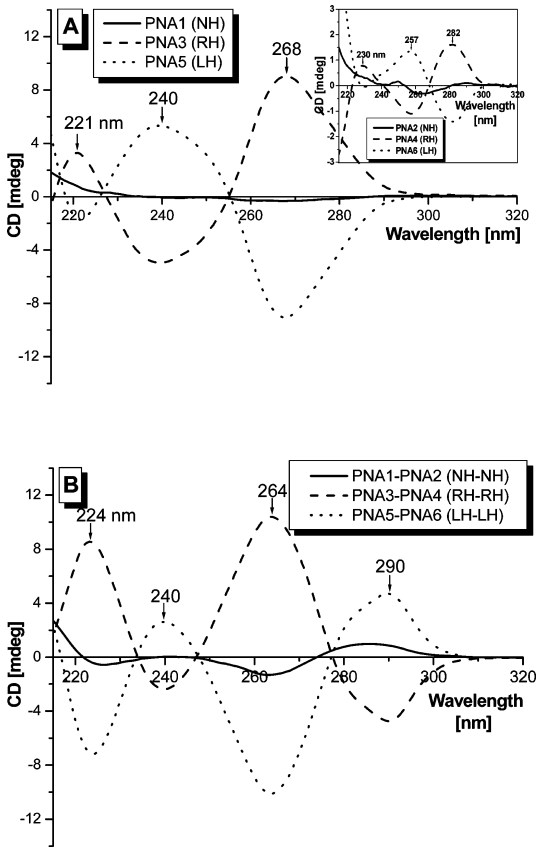

Figure 1. CD spectra of (A) unhybridized (single-stranded) PNA and γPNA oligos, and (B) the corresponding PNA–PNA and γPNA–γPNA duplexes at 5 μM strand concentration each, recorded at 22 °C. *Inset in A:* CD spectra of PNA2, 4, and 6. Otherwise stated, all samples employed in the CD, UV, and fluorescent measurements were prepared in sodium phosphate buffer (10 mM sodium phosphate, 0.1 mM EDTA, 100 mM NaCl, pH 7.2).

plausible explanation for the difference in the CD amplitude is the difference in the degree of base-stacking. It has been reported that thymine has the lowest base-stacking energy among the four nucleobases.[65] This suggestion is consistent with our finding; PNA4 and 6 induced weaker CD signals than PNA3 and 5 because they contained two thymine residues, as compared to none for the latter set. We corroborated this finding with DNA oligonucleotides of the same sequence (Figure S4), confirming the generality of this phenomenon. Interestingly, comparison of the two systems revealed that γPNA oligos produced significantly stronger CD signals than DNA, hinting at greater base-stacking and the structural rigidity of the former. Together these results show that PNA can be directed to fold into an RH or LH helix simply by switching the stereochemistry at the γ-backbone.

An interesting finding is in the comparison of the CD spectra of the individual strands (Figure 1A) to those of the duplexes (Figure 1B). The CD amplitudes of the duplexes are merely the sum of the individual strands. This result supports the notion that, individually, γPNA oligos are already preorganized into the bound state prior to recognition and, as such, hybridization is likely to follow the Fischer's "key and lock" hypothesis.[66] Such binding should translate into higher affinity and sequence selectivity due to the reduction in the entropic penalty and an increase in the backbone rigidity, thereby making the system less accommodating to structural changes. However, in the case of the unmodified PNA1–PNA2 duplex, only weak CD signals were observed, suggesting one of two possibilities: (1) that only a small fraction of the duplex was formed, with the rest in the single-stranded state, or (2) that the duplex existed in nearly equal proportions of RH and LH. As such, the CD signals produced by the RH helix would be canceled by those produced by the LH, hence the weak CD signals. The chirality of the amino acid residue incorporated at the C-terminus has been shown to have no effect on the backbone organization or helical sense of PNA in the single-stranded state (Figure S1); however, it has a small, but notable, effect on the helical preference of the PNA–PNA duplex.[67] On the basis of the CD data alone, it would be difficult to discern one from the other, especially for such a relatively short sequence; however, our UV-melting data discussed in the next section suggest that it is the former.

Thermal and Thermodynamic Stabilities of the Conformationally Matched Homoduplexes. UV-melting experiments were conducted to determine the effect of γ-backbone modifications on the thermal stability of PNA–PNA duplexes. Our results showed that the melting profiles of the conformationally matched γPNA–γPNA duplexes (PNA3–PNA4 and PNA5–PNA6) are nearly identical to each other (Figure 2), with melting transitions (T_m's) of 54 ± 0.5 °C. This

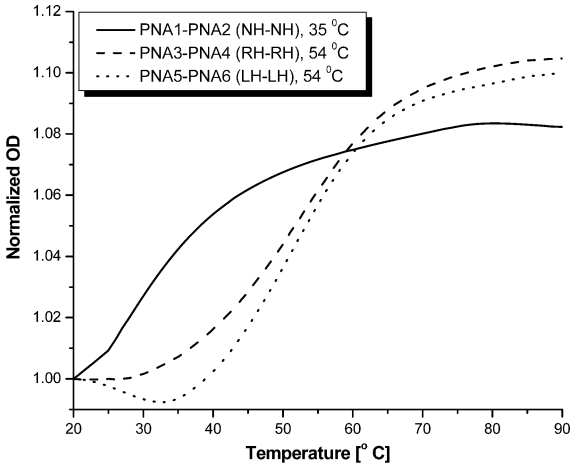

Figure 2. UV-melting profiles of PNA–PNA and γPNA–γPNA duplexes at 5 μM strand concentration each in sodium phosphate buffer.

corresponds to a net gain in ΔT_m of +19 °C, in comparison to that of the unmodified PNA1–PNA2. Concentration-dependent van't Hoff analysis was employed to determine the thermodynamic parameters of the two duplexes: $\Delta H^\circ_{298\ K} = -222 \pm 20$ kJ/mol, $T\Delta S^\circ_{298\ K} = -581 \pm 60$ kJ/mol, and $\Delta G^\circ_{298\ K} = -52 \pm 1$ kJ/mol. The data translate to an average binding free energy of −10.4 ± 0.3 kJ/mol per base pair, in comparison to −6.3 to −8.2 kJ/mol per base pair as previously determined for PNA–PNA.[68−70] We attributed the improvement in thermodynamic stability to backbone preorganization. We had previously attempted to discern the binding free energy gains of γ-backbone modifications, however, to no avail. We did not observe an obvious trend in the entropy term, as would be expected for binding of conformationally preorganized substrates, but instead we noted an entropy–enthalpy compensation.[71] This is because the conformational transformation of PNA is not from a floppy, random-coil to a more compact helical state, but rather from one compact (globular) state to another. PNA adopts a globular motif in order to maximize the solvophobic effect as a result of the charge-neutral backbone and hydrophobic nucleobases.[72−74]

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The difference in the thermodynamic stability of the duplexes, with PNA1−PNA2 being less stable than PNA3−PNA4 or PNA5−PNA6, is consistent with the weaker CD signals observed for the unmodified PNA duplex, indicative of its small fraction present in the solution.

Sequence Specificity. Next, we assessed the ability of γPNA to discriminate between closely related sequences. Comparison of the melting profiles of the single-base mismatched duplexes to that of the perfect match revealed that the destabilization (ΔT_m) is at least −20 °C (Table 2 and Figure S5), which is significantly

Table 2. T_m's of Matched and Single-Base Mismatched Duplexes

Duplex	T_m (°C)	ΔT_m (°C)
PNA5−PNA6	54	
PNA7−PNA6 (G< >T)	34	−20
PNA8−PNA6 (C< >T)	ND	
7PNA9−PNA6 (T< >T)	27	−27

greater than in the case of previously observed PNA and DNA mismatches.[75] The wobble G< >T pair (PNA7−PNA6) was the least destabilizing because such a mismatched pair still retains two H-bonds. Destabilization was more pronounced for the T< >T mismatch (PNA9−PNA6, ΔT_m = −27 °C), and no melting transition was observed for C< >T (PNA8-PNA6), indicating that hybridization did not take place at the indicated temperature range. This result shows that, despite the strong binding affinity, γPNA can discriminate between closely related sequences. We attributed the significant improvement in sequence selectivity to an increase in backbone rigidity and to the greater energetic penalty for each base pair as the result of the shorter sequence.[76]

Recognition Orthogonality. The opposing helical preference of γPNA oligos, RH for PNA3 and 4 and LH for PNA5 and 6, suggested that they might not be able to hybridize to one another despite the sequence complementarity. Such an inherent property would be valuable, if it could be demonstrated, for programming molecular assembly because of the added dimension in recognition. To determine if recognition orthogonality was in play between RH and LH, we measured the thermal stabilities of the conformationally mismatched pairs (PNA3−PNA6 and PNA4−PNA5) and compared them to that of the matched γPNA−γPNA homoduplexes (PNA3−PNA4 and PNA5−PNA6). Since the melting profiles of the homoduplexes were shown to be nearly identical to each other (Figure 2), only one was chosen for comparison. Inspection of Figure 3 reveals that the thermal profiles of the conformationally mismatched PNA3−PNA6 (RH−LH) and PNA4−PNA5 (RH−LH) pairs have no discernible transitions. Rather, they closely resemble that of the sum of the individual strands (Figure 3, *Inset*), indicating that these conformationally mismatched pairs did not hybridize to each other. We attributed the residual hyperchromicities to the melting (or unstacking) of the nucleobases within each strand.

To independently verify the recognition orthogonality of the conformationally mismatched γPNA oligos, we performed fluorescent experiments using pyrene as a reporter probe. Pyrene has been used as a fluorescent marker to investigate the conformational arrangements of proteins and nucleic acids, where formation or disruption of the pyrene excimer is indicative of folding or denaturation.[77−80] When excited at 344 nm, the pyrene monomers emit fluorescent signals at 380 and 400 nm, but when they are stacked with each other to form dimers, their

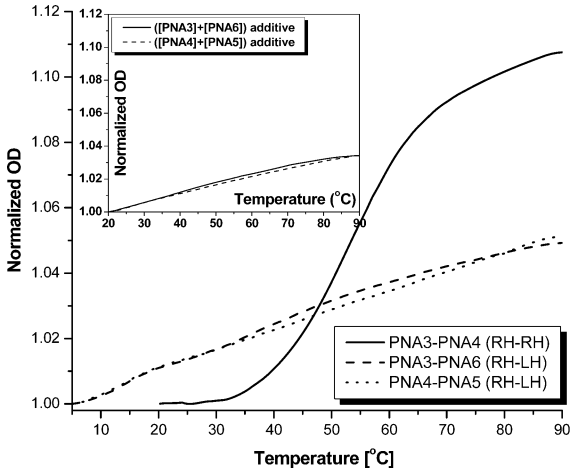

Figure 3. UV-melting profiles of γPNA−γPNA duplexes containing conformationally matched (RH−RH) and mismatched (RH−LH and LH−RH) helical senses at 5 μM strand concentration each in sodium phosphate buffer.

excitation results in the formation of excimers which emit at 485 nm. This ∼100 nm red shift in the fluorescent emission provides a convenient means for monitoring the on and off state of molecular interactions. By attaching pyrene to the terminal regions of the complementary γPNA pairs, one at the C- and the other at the N-terminus (Figure 4A), one could easily determine if hybridization takes place by monitoring the pyrene emissions. In the absence of hybridization, the pyrenes on individual γPNA strands exist as monomers, and as such, their excitation at 344 nm should produce blue fluorescent emissions at 380 and 400 nm (Figure 4B). However, upon hybridization, whereby the two complementary γPNA strands come together and the opposing pyrene ligands are stacked with each other to form dimers, excitation at the same wavelength (344 nm) should produce excimers which emit fluorescence signals at 485 nm. The large shift in the emissions should enable detection even with the naked eye.

The samples were prepared by mixing equimolar concentrations of the various γPNA pairs, conformationally matched as well as mismatched, and annealing at 95 °C for 5 min. The mixtures were excited at 344 nm, and the fluorescent emissions were recorded from 340 to 600 nm at room temperature. As expected, the samples with a complementary sequence and conformationally matched pairs (PNA3P−PNA4P and PNA5P−PNA6P) showed distinct pyrene excimer emissions at 485 nm, with residual monomer emissions at 380 and 400 nm (Figure 4C). The excimer emissions indicated that the two strands hybridized to each other. We attributed the residual monomer emissions to the small fraction of the single strands present in the solution in equilibrium with the duplex. In contrast, the conformationally mismatched pairs (PNA3P−PNA6P and PNA4P−PNA5P) showed only the monomer emissions, indicating the absence of hybridization. Figure 4D shows a photograph of the four samples irradiated with a short-wavelength hand-held UV-lamp, confirming the recognition orthogonality of the conformationally mismatched pairs. This result is consistent with the UV and CD measurements, indicating that γPNA strands with a complementary sequence, but opposing helical sense, do not hybridize to each other.

Hybridization of RH- and LH-γPNA to RNA and DNA. Next, we assessed the ability of RH- and LH-γPNA to hybridize

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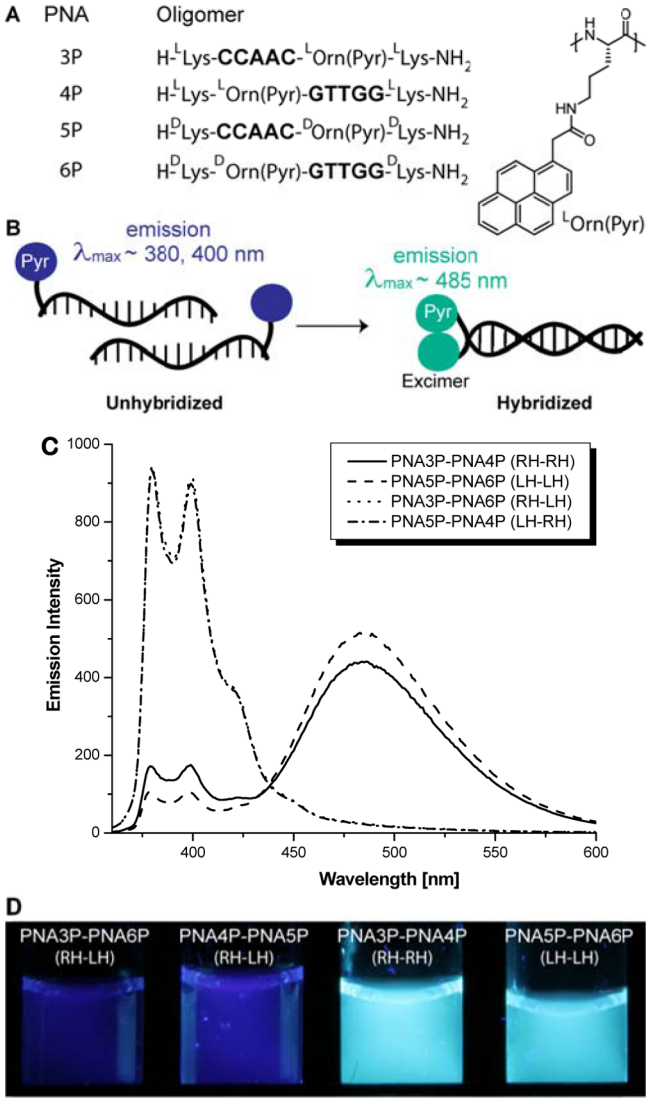

Figure 4. (A) Sequence of γPNA oligos employed in the fluorescent measurements, along with the structure of pyrene to which they were covalently linked. (B) Schematic diagram showing the expected emissions of pyrene in γPNA oligos in the hybridized and unhybridized states. (C) Fluorescent spectra of the complementary γPNA strands containing conformationally matched (RH−RH and LH−LH) and mismatched (RH−LH and LH−RH) helical senses following excitation at 344 nm. (D) Photographs of the samples employed in (C) under a hand-held UV-lamp.

to complementary DNA or RNA strand. UV-melting data revealed that, similar to the observations made with the γPNA−γPNA homoduplexes, RH-γPNA oligos (PNA3 and PNA4) were able to hybridize to complementary DNA (Figure 5) as well as RNA strands (*Inset*), as evidenced from the sigmoidal profiles and clear melting transitions. In line with the previous observation, we found the thermal stabilities of the RH-γPNA−RNA duplexes to be 5−10 °C higher than that of RH-γPNA−DNA.[71] In contrast, the conformationally mismatched LH-γPNA−DNA (PNA5−DNA1 and PNA6−DNA2) and LH-γPNA−RNA (PNA5−RNA1 and PNA6−RNA2) pairs did not show the two-state melting behaviors, indicating that LH-γPNA oligos did not hybridize to the complementary DNA or RNA strands. This result suggests the possibility of using LH-γPNA to organize molecular self-assembly and carry out molecular

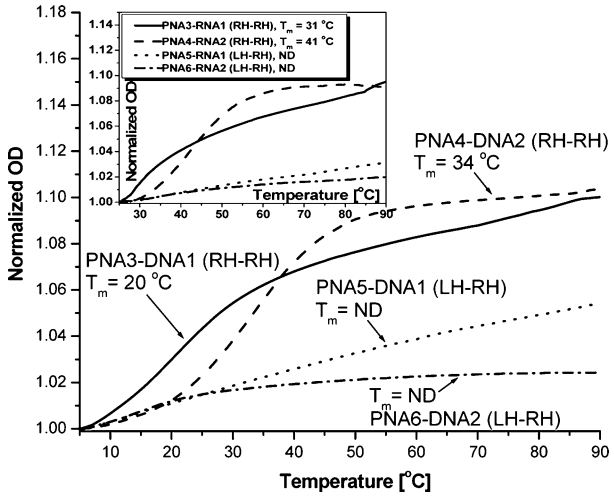

Figure 5. UV-melting profiles of γPNA−DNA pairs with matched (RH−RH) and mismatched (LH−RH) helical sense at 5 μM strand concentration each. *Inset:* melting curves of γPNA−RNA pairs. DNA1:5′-GTTGG-3′, DNA2:5′-CCAAC-3′, RNA1:5′-GUUGG-3′, RNA2:5′-CCAAC-3′.

computation *in vivo* without the concern of cross-hybridization with the endogenous genetic materials, or enzymatic degradation, since γPNA is non-natural and, therefore, should be impervious to recognition by proteases or nucleases.[81]

Dual Recognition of PNA with RH- and LH-γPNA. We have demonstrated that neither RH- and LH-γPNA oligos were unable to hybridize to each other and nor were LH-γPNAs to complementary RNA or DNA strands, due to conformational mismatch. Since PNA is achiral and does not have a well-defined conformation, we suspected that it might be able to hybridize to LH as well as RH-γPNA. Such capability is valuable for translating the genetic information encoded in one conformer to another. Figure 6 shows the UV-melting profiles of PNA1 and 2 after hybridization with the complementary LH- and RH-γPNA strands. Their inspection reveals that unmodified (achiral) PNA is able to hybridize to both the LH and RH conformers. The fact that the melting profiles of PNA1−PNA4 and PNA1−PNA6 are virtually identical to each other ($T_m \approx 43$ °C), and likewise

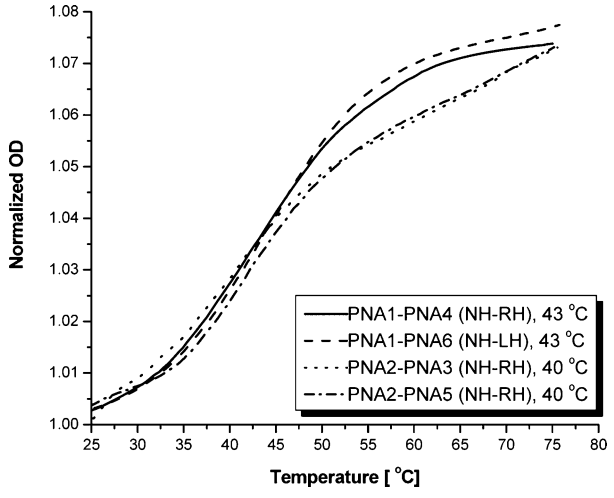

Figure 6. UV-melting profiles of PNA−γPNA heteroduplexes, with the latter containing RH and LH helical motifs at 5 μM strand concentration each.

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PNA2-PNA3 and PNA2-PNA5 ($T_m \approx 40$ °C), indicates that PNA has an equal propensity to hybridize to LH- as well as RH-γPNA. A slight variation in the thermal stability between the two sets was expected because of the inversion in the sequence. The ability of PNA to interface with LH- and RH-γPNA, as well as with DNA and RNA, makes it a versatile platform for storage and transmission of genetic information, one that is compatible with genetic materials, as well as one that is orthogonal to them. One such potential application is *in situ* detection of genetic materials based on the HCR technology developed by Pierce and co-workers.[31,82,83]

Molecular Self-Assembly. To demonstrate the feasibility and recognition orthogonality of the pentameric γPNA system in organizing molecular self-assembly, we employed two sets of polystyrene beads, 2 and 10 μm in size, with the surface coated with γPNA via biotin−streptavidin binding (Figure 7A). To circumvent the possible collapse of relatively hydrophobic Me-γPNA oligos onto the surface of the polystyrene beads, we replaced the Me-group with diethylene glycol (DEG) at the γ-backbone, which we had previously shown to significantly improve water solubility and biocompatibility.[71] Additionally, a long, flexible $(Mpeg)_5$ linker was inserted between the recognition module and biotin to provide greater conformational flexibility and ease of hybridization. The biotin and fluorescent probes, with the latter used to mark the helical sense of γPNA oligos, were covalently attached to the ornithine side chain. Figure 7B shows a scheme of the four types of polystyrene beads employed in the study. The assembly process was initiated by mixing equimolar concentrations of beads containing different combinations of conformationally matched and mismatched γPNA oligos, with the resulting DIC images shown in Figure 7C after annealing at room temperature for 3 h. The results revealed that only polystyrene beads containing γPNA oligos with a complementary sequence and matching helical sense were able to interact with each other and form the expected concentric "small-on-large" bead arrangements [Figure 7C, images (i) and (iv)]. The insets in images (i) and (iv) further confirmed the helical sense of γPNA oligos, as indicated by the color of the fluorescent probe. However, in the case of γPNA oligos with mismatched helical senses, no defined bead interactions were observed [images (ii) and (iii)], indicating that they did not recognize one another due to conformational mismatch; therefore, the 2 μM beads were not able to interact with and self-organize around the larger ones, despite the sequence complementarity. This result confirms the findings from the CD and UV-melting experiments, demonstrating the recognition orthogonality of LH- and RH-γPNA. To the best of our knowledge, this is the first example of a pentameric recognition module capable of organizing molecular self-assembly.

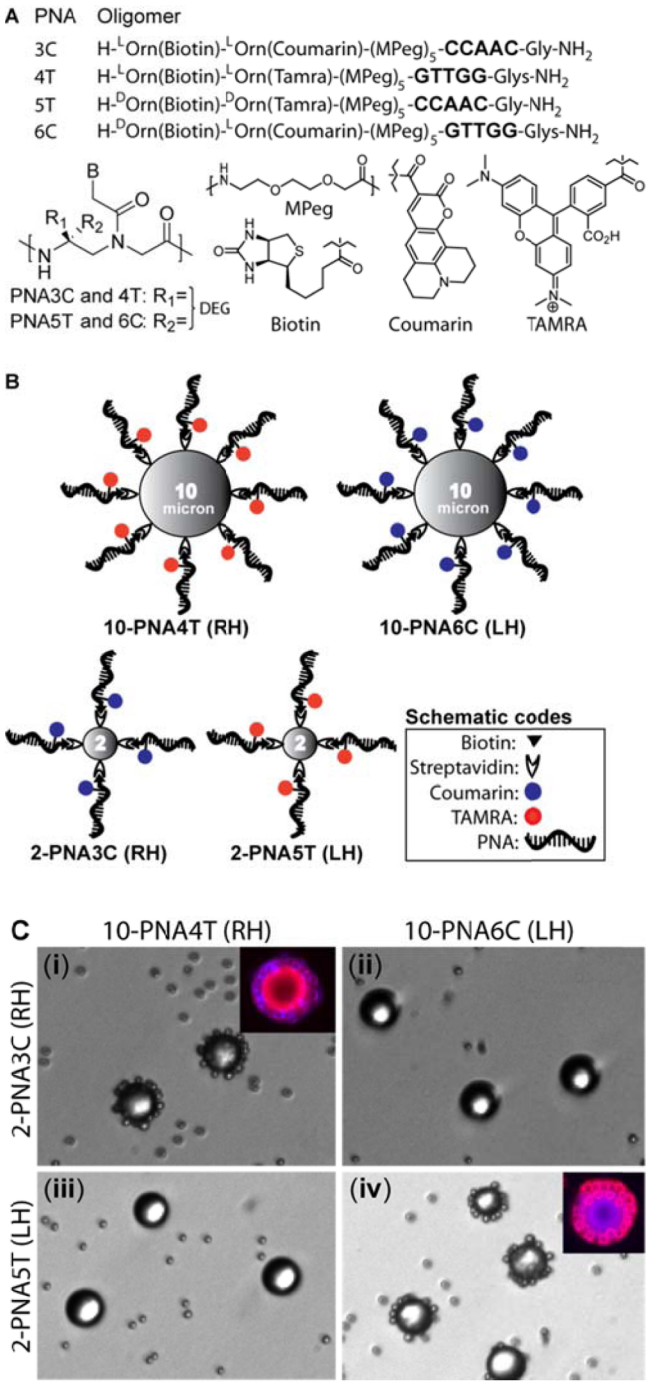

Figure 7. (A) Sequence of γPNA oligos utilized in the assembly of polystyrene beads along with chemical structures of the various constituents. (B) Schematic diagram of streptavidin-coated polystyrene beads labeled with the indicated γPNA oligos through streptavidin−biotin binding. The naming system is as follows: bead size (2 or 10 μm), oligomer name (PNA3, 4, 5, or 6), fluorescent probe (C: Coumarin, T: Tamra), and helical sense in the parentheses (RH or LH). For instance, 10-PNA4T (RH) stands for a polystyrene bead 10 μm in size, covalently coated with PNA4, marked by a Tamra (red) fluorescent probe, with the recognition code adopting an RH helical sense. (C) DIC images of different combinations of polystyrene bead mixtures. *Insets* in (i) and (iv): fluorescent images of the assembled polystyrene beads.

CONCLUSIONS

In summary we have demonstrated that PNA, which, as an individual strand, does not have a well-defined conformation in solution, can be preorganized into an RH or LH helical motif simply by installing an appropriate stereogenic center at the γ-backbone. LH- and RH-γPNA oligos hybridize to their partner strands containing a complementary sequence and matching helical sense; however, they do not cross-hybridize with one another. Binding occurs with unusually high affinity and sequence selectivity, presumably through the "key and lock" motif with minimal conformational rearrangement as the result of backbone preorganization. Recognition modules, as short as 5 nts in length, can be used to organize and assemble micro-particles with a high level of recognition orthogonality. Likewise, due to conformational mismatch, LH-γPNA oligos are unable to hybridize to complementary DNA or RNA strands. The

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recognition orthogonality of LH- and RH-γPNA can be interfaced with achiral PNA as well as with DNA and RNA, providing an "all-in-one" nucleic acid platform for programming molecular interactions and assembly.

The utility of PNA in molecular self-assembly and programming chemical reactivity has been demonstrated by Liu,[84] Seitz,[85] and Winssinger.[86] The added dimension in recognition orthogonality, combined with the superior binding affinity and sequence selectivity along with the ease of chemical synthesis and functional group diversification, will certainly expand the utility of nucleic acid based molecular engineering and computing, both *in vitro* and *in vivo*. The relatively small size of the recognition module (5 to 8 nts in length) should be easy and cost-effective to synthesize in high-throughput and to scale-up. Moreover, the reduction in size should make cell delivery more manageable and efficient, in comparison to the longer traditional antisense reagents, when functionalized with the appropriate chemical groups or employing an appropriate delivery vehicle.[87] In addition to the LH and RH conformational orthogonality, another dimension of recognition orthogonality that could be implemented is base pairing. Inclusion of unnatural base pairs, such as isoC/isoG and others,[88,89] that do not recognize the natural nucleobases will expand the recognition repertoire of such a system further, providing greater flexibility and a greater level of orthogonality in programming molecular interaction, for organization and assembly of materials as well as for molecular computing.

■ ASSOCIATED CONTENT

Supporting Information

Chemical structures of the monomers; UV and CD spectra of various DNA, PNA, and γPNA oligos in the single-stranded and double-stranded states; and experimental procedures. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.5b04566.

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Notes

The authors declare no competing financial interest.

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