Electronic Supplementary Material (ESI) for Chemical Communications.

Supplementary Information

Cationic Guanine: Positively Charged Nucleobase with Improved DNA Affinity Inhibits Self-Duplex Formation

Masaki Hibino, Yuichiro Aiba*, Osami Shoji*

Department of Chemistry, Graduate School of Science, Nagoya University,
Furo-cho, Chikusa-ku, Nagoya, Aichi 464-8601, Japan

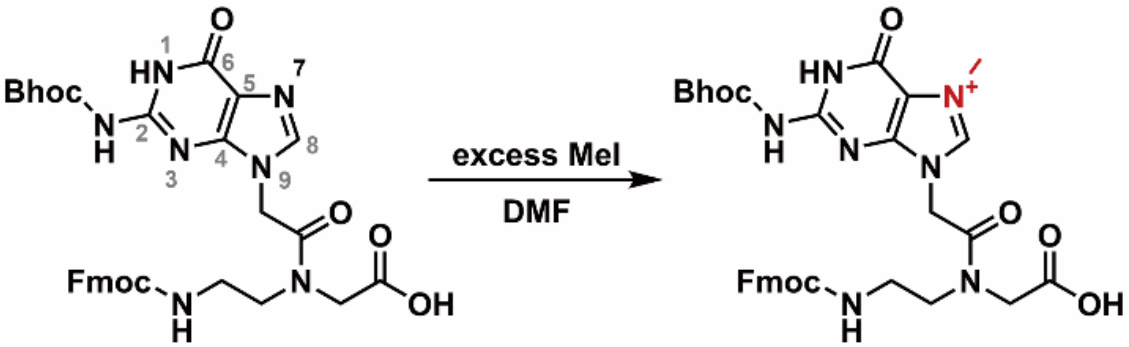
Experimental Section

Materials

Solvents and reagents were purchased from Sigma-Aldrich, Wako, TCI, Kanto Chemical, or nakalai tesque and used as received without further purification. PNA monomers were purchased from PANA GENE, and ASM Research Chemicals. Oligonucleotides were purchased from Fasmac. All manipulations of air-sensitive materials were carried out under an atmosphere of dry argon by standard Schlenk techniques.

PNA incorporated with pseudo-complementary bases were synthesized by standard Boc-chemistry-based solid phase peptide synthesis according to a literature procedure,[1] and the other PNA were synthesized by Fmoc method with an automated peptide synthesizer (Biotage, Syro I). All PNAs were purified by reversed-phase HPLC and characterized by MALDI-TOF MS (Bruker Daltonics, ultraflex III) using sinapic acid as a matrix.

Synthesis of Fmoc cationic guanine (G^+) PNA monomer



Scheme S1. Synthetic procedure of cationic guanine PNA monomer.

100 mg (135 μmol) of commercially available Fmoc-PNA(G)-OH was dissolved in 1 mL of dehydrated DMF under argon atmosphere, and 168 μL (2.70 mmol, 20 eq.) of iodomethane was added dropwisely. The reaction mixture was stirred at room temperature for 7.5 h and slowly turned yellow. After reaction, *ca*. 30 mL of diethyl ether was poured into the reaction mixture, and the solvent was kept at 4 °C overnight. The supernatant was carefully removed by decantation, and orange transparent oil was obtained. Followed by addition of 1 mL of water, yellowish-white precipitate was obtained by sonication and scratching with spatula. The precipitate was collected by centrifugation and washed with water and diethyl ether to give the white solid, Yield 101 mg (98%). The product was identified by ESI-TOF MS and ^{1}H NMR.

ESI-TOF MS (m/z): calcd $[M]^+$ 756.2776, found 756.3106

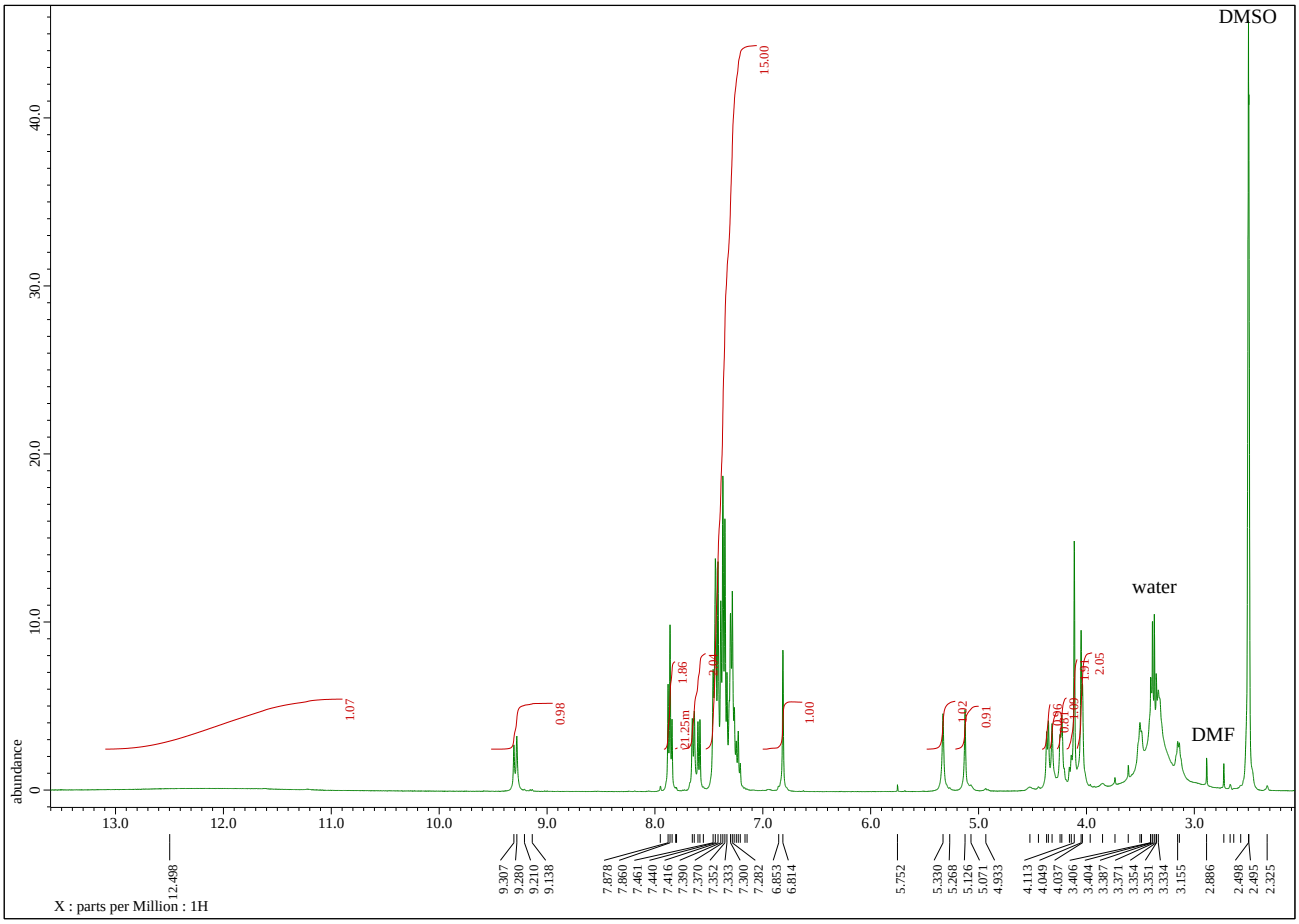


Figure S1. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.05−3.60 (m, water and *n*H) 4.04 (d, 2H), 4.11 (m, 2H), 4.23 (d, 1H), 4.32 (s, 1H), 4.36 (d, 1H), 5.13 (s, 1H), 5.33 (s, 1H), 6.81 (s, 1H), 7.21−7.46 (m, 15H), 7.57−7.67 (dd, 2H), 7.86 (t, 2H), 9.29 (d, 1H), 12.50 (br. s, 1H) ppm. Peaks at 7.81 ppm (d, 1H) of reactant disappeared and moved to 9.29 ppm by methylation.

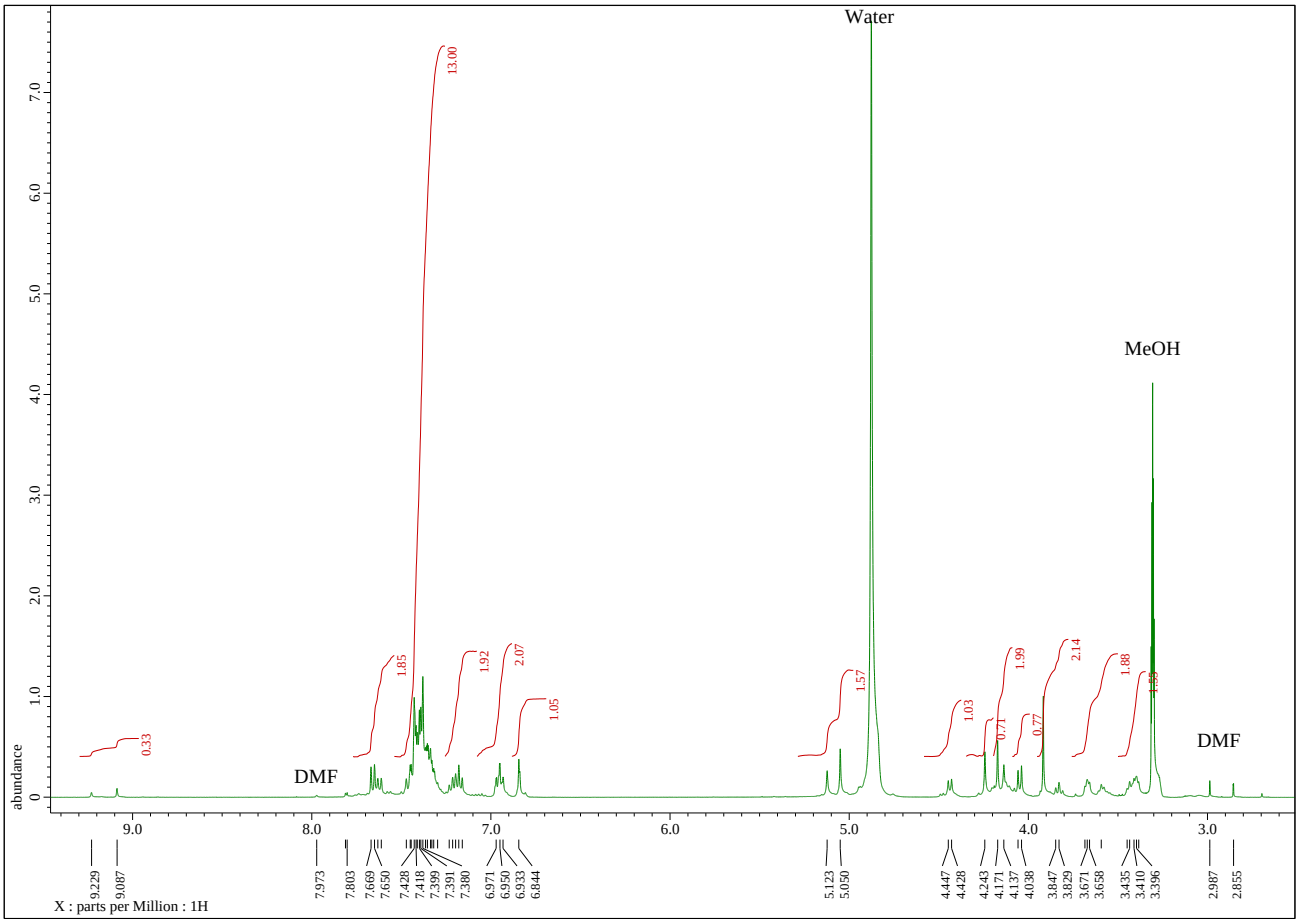


Figure S2. ^{1}H NMR (400 MHz, CD_3OD): δ 3.37−3.43 (m, 1H), 3.57−3.71 (tt, 2H), 3.81−3.94 (m, 2H), 4.05 (d, 1H), 4.11−4.19 (m, 2H), 4.24 (s, 1H), 4.43 (d, 1H), 5.09 (d, 2H), 5.48 (s, 1H), 6.84 (s, 1H), 6.95 (m, 2H), 7.18 (m, 2H), 7.3−7.5 (m, 13H), 7.60-7.67 (dd, 2H), 9.08-9.22 (d, 1H).

T_m measurements

Melting temperatures (T_m) of the PNA/PNA and PNA/DNA duplexes were estimated from UV melting curves measured on a JASCO V-730 BIO Spectrophotometre. A solution of 10-mer PNA was prepared with its complementary PNA or DNA (1 μM) in 5 mM HEPES buffer (pH 7.0) under physiological salt conditions ([NaCl] = 12 mM, [KCl] = 139 mM, and [$MgCl_2$] = 0.8 mM). Samples were denatured by prior incubation at 90 °C for several minutes, and their change in absorbance was monitored at 260 nm by decreasing and increasing the temperature between 90 °C and 25 °C at a rate of 0.2 °C/min. Each measurement was repeated at least twice, with the T_m value representing the average value of the inflection point.

Determination of invasion efficiency

Target 130-bp DNA from pBR322 (50 nM, T1776–G1905 region) was incubated with a pair of complementary PNAs (250–300 nM, targeting the C1826–C1835 region) in 5 mM HEPES buffer (pH 7.0) at 37 °C for 12 h. After incubation, the solutions were subjected to microchip electrophoresis (SHIMADZU, MultiNA), and invasion efficiency was evaluated by electrophoretic mobility shift assay. The invasion efficiency was calculated as follows:

(Invasion efficiency) = (Band intensity of invasion complex) / (Sum of all band intensities)

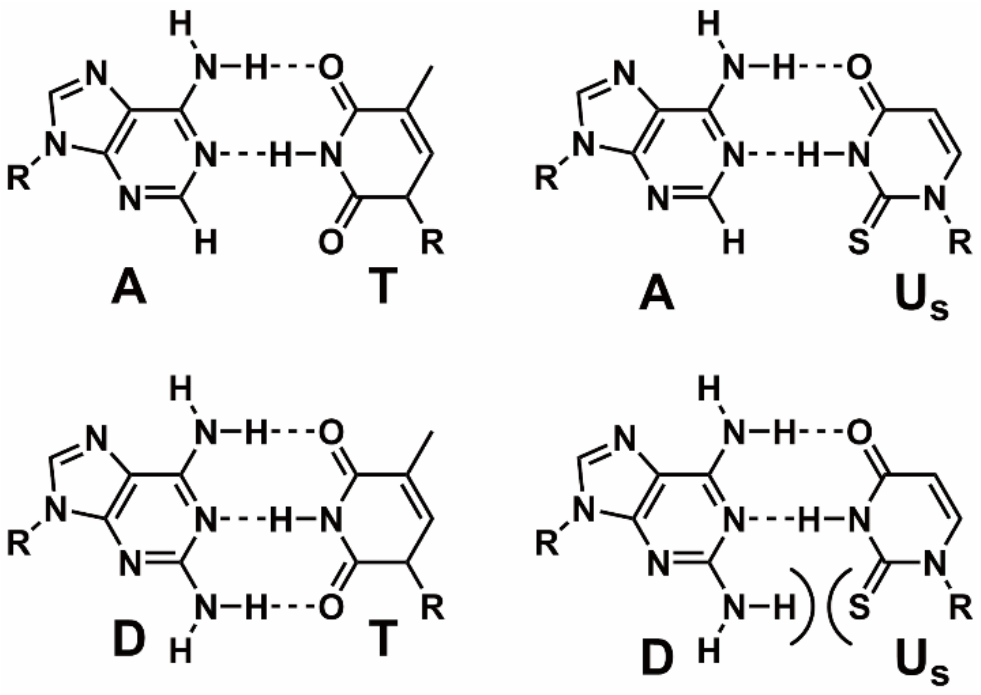


Figure S3. Base pairs between pseudo-complementary nucleobases (D, U_s) and natural nucleobases (A, T).

Stability of G^+-PNA

Methylation at the N^7-position of guanine also occurs in nature, but it is well known that N^7-methylguanine DNA is susceptible to depurination and swiftly degraded.[2, 3] To confirm the stability of G^+-PNA, PNA^+-Rev was incubated in 5 mM HEPES buffer (pH 7.0) at 37 °C for up to 66 h. A solution containing G^+-PNA was analysed by HPLC at different intervals. It was reported that 30% of N^7-methyldeoxyguanosin is degraded within 30 h,[4] but PNA^+-Rev seemed to be stable even after incubation for 66 h (Fig. S2). Based on this result, G^+-PNA, unlike N^7-methyldeoxyguanosin, is sufficiently stable under our experimental conditions.

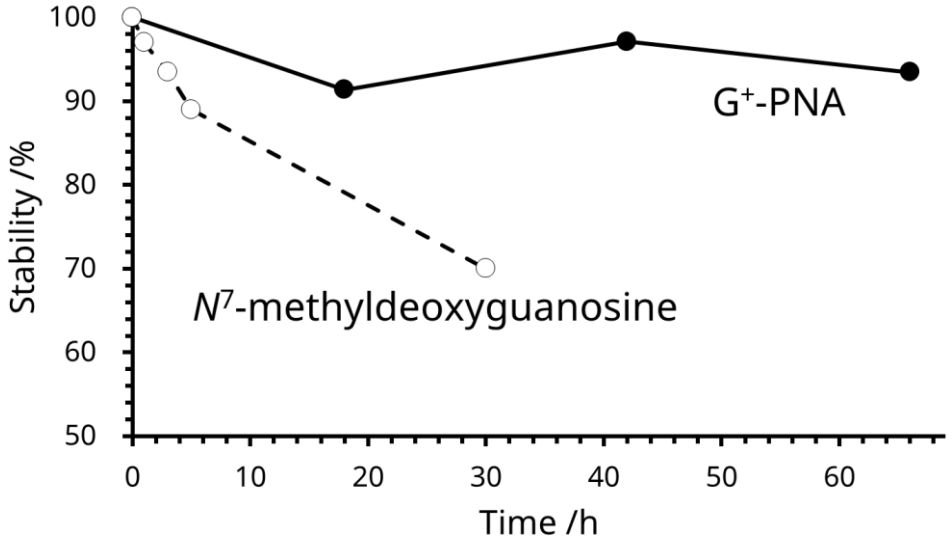


Figure S4. Stability of N^7-methyldeoxyguanosin (dashed line; values taken from a previous report[4]) and PNA^+-Rev (solid line; this research).

Table S1. Sequences of PNAs employed in this study.

Name	Sequence of 10-mer PNA (N to C)†
PNA-Fw	KGTTACTGATGKK
PNA^{+}-Fw	KGTTACT$\overset{+}{G}$ATGKK
PNA^{3+}-Fw	K$\overset{+}{G}$TTACT$\overset{+}{G}$AT$\overset{+}{G}$KK
pcPNA-Fw	KGU$_s$U$_s$DCU$_s$GDU$_s$GKK
$pcPNA^{+}$-Fw	KGU$_s$U$_s$DCU$_s$$\overset{+}{G}DU_s$GKK
$pcPNA^{3+}$-Fw	K$\overset{+}{G}$U$_s$U$_s$DCU$_s$$\overset{+}{G}DU_s$$\overset{+}{G}$KK
PNA-Rev	KCATCAGTAACKK
PNA^{+}-Rev	KCATCA$\overset{+}{G}$TAACKK
pcPNA-Rev	KCDU$_s$CDGU$_s$DDCKK
$pcPNA^{+}$-Rev	KCDU$_s$CD$\overset{+}{G}$U$_s$DDCKK

† K = lysine; G^{+} = cationic guanine; D = 2,6-diaminopurine; U_s = 2-thiouracil.

Table S2. Sequences of PNAs employed in this Supplementary Information.

Name	Sequence of 10-mer PNA (N to C)†
PNA^{+}-Fw(N)	K$\overset{+}{G}$TTACTGATGKK
PNA^{+}-Fw(C)	KGTTACTGAT$\overset{+}{G}$KK

† K = lysine; G^{+} = cationic guanine.

Table S3. Sequences of DNA.

Name	Sequence of 10-mer DNA (5' to 3')†
DNA-Fw	GTTACTGATG
DNA'-Fw	GTTACT***C***ATG
DNA-Rev	CATCAGTAAC
DNA'-Rev	CAT***G***AGTAAC

† Bold italic: mismatch. C1826–C1835 region of pBR322.

Position Dependence of Introduced G^+

The DNA affinity and self-avoiding ability of N- or C-terminal modified G^+-PNA (Table S2) were evaluated by T_m analysis (Fig. S5).

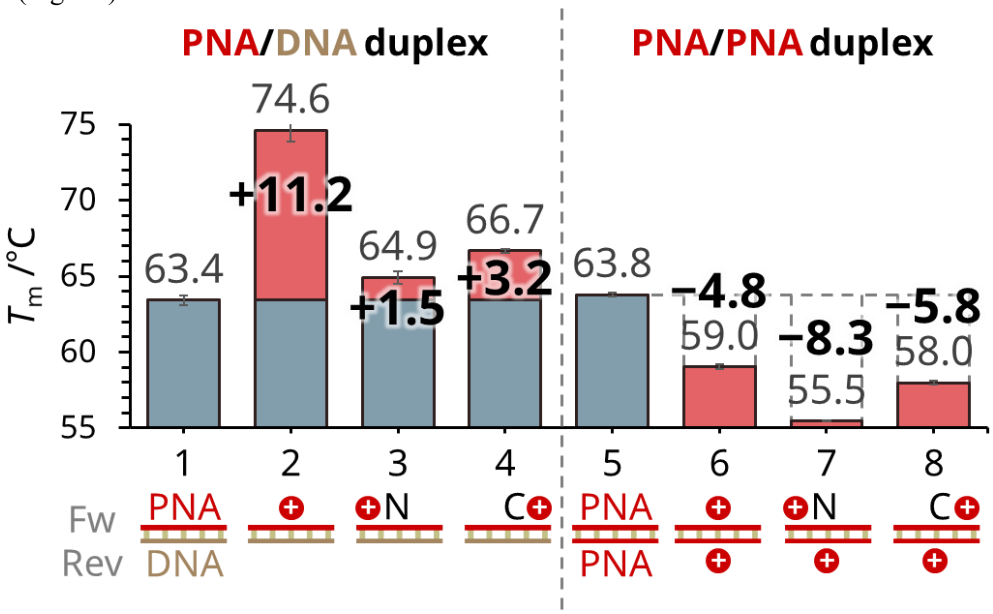


Figure S5. Effect of G^+-position on the affinity and self-avoiding property of G^+-PNA. PNA/DNA (bars 1–4) and PNA/PNA (bars 5–8) duplexes. T_m values are displayed above their corresponding bars. Bar 1, PNA-Fw/DNA-Rev; bar 2, PNA^+-Fw/DNA-Rev; bar 3, PNA^+-Fw(N)/DNA-Rev; bar 4, PNA^+-Fw(C)/DNA-Rev; bar 5, PNA-Fw/PNA-Rev; bar 6, PNA^+-Fw/PNA^+-Rev; bar 7, PNA^+-Fw(N)/PNA^+-Rev; bar 8, PNA^+-Fw(C)/PNA^+-Rev. Conditions: [each DNA and PNA] = 1 μM, [HEPES (pH 7.0)] = 5 mM.

Invasion complex incorporating a mismatch

Figure S6. Sequences of PNAs and target double-stranded DNA incorporating a mismatch. Bold characters in the DNA sequences represent mismatched bases.

Mismatch Discrimination Ability of G$^+$-PNA

Because oligonucleotides with positive charges frequently cause nonspecific binding to negatively charged DNA,[5] the sequence specificity of G$^+$-PNA was evaluated by measuring T_m values with mismatch target DNAs (Fig. S7a). Initially, PNA$^+$-Fw and PNA$^+$-Rev were used for the T_m measurement, but they did not show definite two-state transition curves with mismatched target DNAs, meaning that those PNA/DNA duplexes containing a mismatch were too unstable to form stable duplexes under the measurement conditions (25–90 °C).

To evaluate mismatch discrimination ability of G$^+$-PNA precisely, pcPNA$^+$-Fw and pcPNA$^+$-Rev (Table S1), whose DNA affinity was improved by the introduction of pseudo-complementary nucleobases D and Us, were used to facilitate the evaluation. When pcPNA containing no cationic guanine was employed, T_m between pcPNA-Fw and its fully complementary target (DNA-Rev) was 52.9 °C (Fig. S7b, bar 1), and T_m between pcPNA-Fw and single-base mismatched target (DNA'-Rev) was 30.8 °C (bar 3). Thus, the introduction of single-base mismatch decreased T_m of pcPNA-Fw by 22.1 °C. On the other hand, a single-base mismatch decreased, the T_m of pcPNA$^+$-Fw, which contain cationic guanine, by 26.7 °C (bars 2, 4; from 60.2 °C to 33.5 °C). This value is larger than that of pcPNA-Fw (22.1 °C). The same tendency was also observed for pcPNA$^+$-Rev and pcPNA-Rev (bars 5–8). Consequently, it was concluded that the introduction of cationic guanine does not lower the mismatch discrimination ability of PNA but rather slightly improved it.

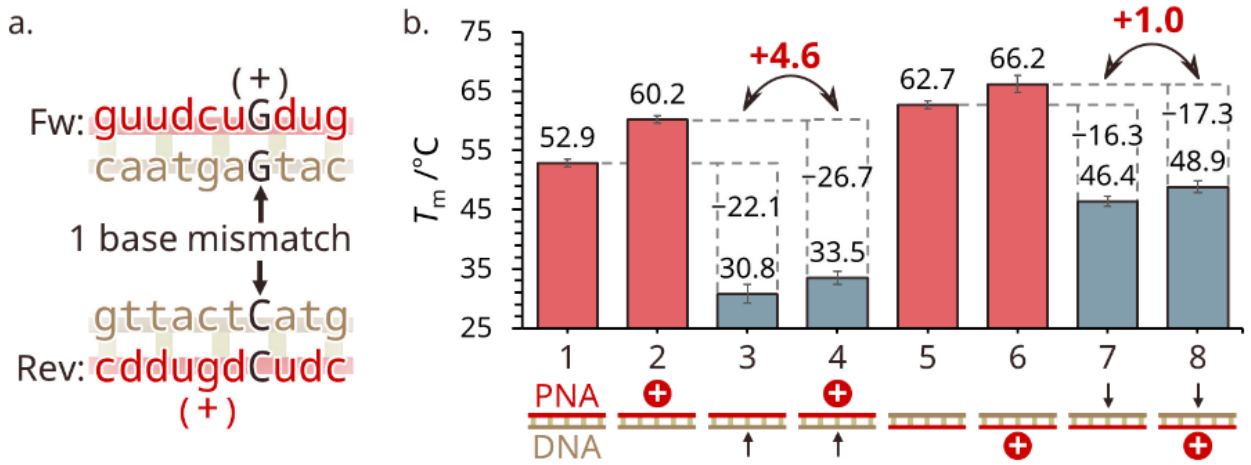


Figure S7. (a) Sequences of PNAs and complementary DNA containing a mismatch base pair. Black upper case, d and u stand for mismatched base pair, 2,6-diaminopurine, and 2-thiouracil, respectively. (b) T_m measurements of PNAs with full-matched DNAs (red; bars 1, 2, 5, and 6) and mismatched DNAs (blue bars 3, 4, 7, and 8) under physiological conditions. Bars 1,3: pcPNA-Fw; bars 2,4: pcPNA$^+$-Fw; bars 5,7: pcPNA-Rev; bars 6,8: pcPNA$^+$-Rev. Conditions: [DNA] = [PNA] = 1 μM, [HEPES (pH 7.0)] = 5 mM, [NaCl] = 12 mM, [KCl] = 139 mM, and [$MgCl_2$] = 0.8 mM.

Effect of salt upon the self-avoiding property of G$^+$-PNA

When PNA are used under physiological conditions, such as *in cellula*, they are exposed to high salt concentrations. Salt, especially at high concentrations, is known to interfere negatively with electrostatic interactions, making it imperative to evaluate the effect of salt upon the self-avoiding ability of G$^+$-PNA. The difference in melting temperature (ΔT_m) between PNA-Fw/DNA-Rev and PNA$^+$-Fw/DNA-Rev (Fig. S9a), and between PNA-Fw/PNA-Rev and PNA^{3+}-Fw/PNA$^+$-Rev (Fig. S9b) were plotted against the NaCl concentration. Although, with increasing salt concentration, the absolute values of ΔT_m of PNA/DNA and PNA/PNA duplexes indeed decreased, meaning the electrostatic interaction of G$^+$-PNA was weakened under high salt conditions, self-avoiding ability as well as DNA binding affinity was still higher than that of corresponding unmodified PNAs even at 100 mM NaCl (ΔT_m > 3 °C). These results indicate that electrostatic attraction and repulsion mainly contribute to the self-avoiding ability of G$^+$-PNA, as intended, and cationic guanine is anticipated to be applicable under physiological conditions.

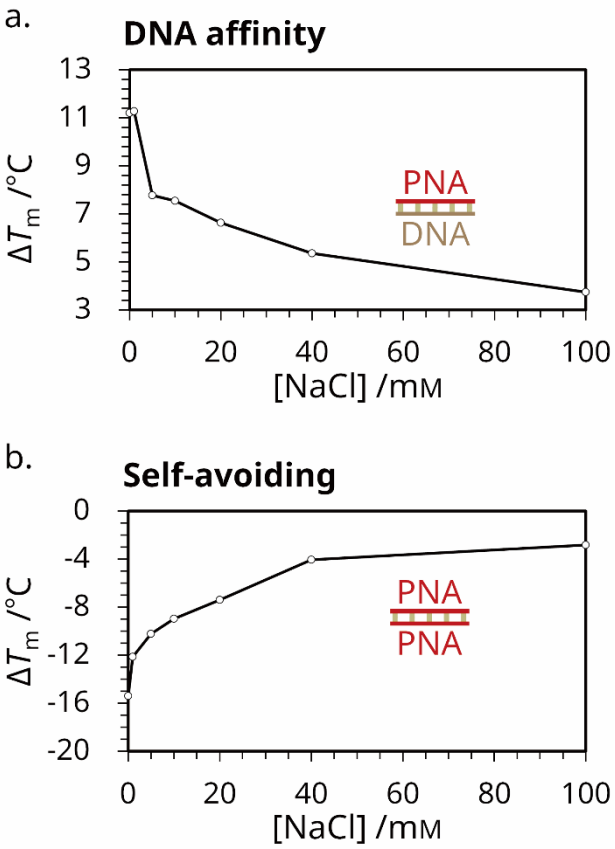


Figure S8. Effect of NaCl on the DNA affinity and self-avoiding property of G$^+$-PNA. (a) ΔT_m between PNA-Fw/DNA-Rev and PNA$^+$-Fw/DNA-Rev; (b) ΔT_m between PNA duplexes (PNA-Fw/PNA-Rev and PNA^{3+}-Fw/PNA$^+$-Rev). Conditions: [each DNA and PNA] = 1 μM, [HEPES (pH 7.0)] = 5 mM, and [NaCl] = 0–100 mM.

Reference

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