Supplementary Informaiton

Introduction of disulfide bond to the main chain of PNA to switch its hybridization and invasion activity

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The preparation of DNAs for the DNA separation experiment.

The 915 bp non-target DNA was prepared by PCR. The reaction mixture (150 μl) contained 30 ng of template DNA, 2.5 U of *TaKaRa Ex Taq*® DNA polymerase (TaKaRa), *Ex Taq* buffer, 1.0 μM of each primer and 0.2 mM of each dNTP (the cycle: 30 sec at 94 ^{o}C, 30 sec at 55 ^{o}C and 30 sec at 72 ^{o}C for 30 cycles). The DNA products were purified by QIAquick PCR Purification Kit (QIAGEN).

The 4203 bp target DNA was prepared from pBR322 plasmid DNA (TaKaRa). First, pBR322 DNA was digested by *Hin*d III (TaKaRa) and *Eco*R V (TaKaRa) and then dephosphorylated by rAPid Alkaline Phosphatase (Roche). To remove the smaller fragment (~ 150 bp) produced by double-digestion, the DNA solution was subjected to S-400 MicroSpin Columns (GE Healthcare UK Ltd.). The resultant long DNA (~ 4200 bp) has a blunt end and sticky end (-TCGA-5'). To incorporate the 13-mer overhang region which is complementary to PNA-S3 (and PNA-3), 5'-phosphorylated 13-mer oligonucleotide (5'-pAGCTCCCGTATCGTGAG-3') was ligated. The 4.2-kbp DNA was mixed with 200-fold 13-mer oligonucleotide and then the ligation was achieved by DNA Ligation Kit Ver.2.1 (TaKaRa). All purification steps were done by using QIAquick PCR Purification Kit.

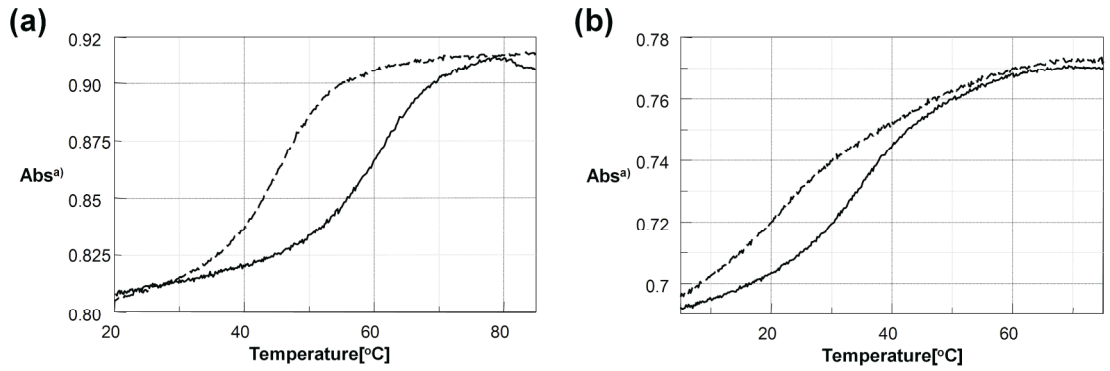

Fig. S1 Melting curves of the duplexes of PNA-1 (a) or PNA-S1 (b) with its mismatched 10-mer DNA (DNA-M1 (the solid line) or DNA-M2 (the broken line)). The absorbance at 260 nm was measured under these conditions: [PNA] = 3.0 μM, [DNA] = 3.0 μM, and [NaCl] = 100 mM in pH 7.0 HEPES buffer (5.0 mM).

[a)] The absorbance was normalized to facilitate the comparison.

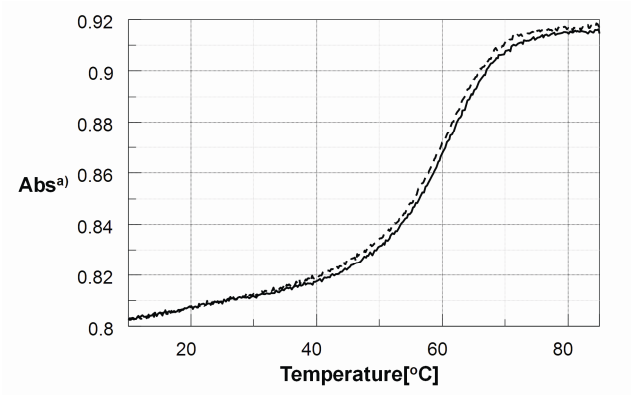

Fig. S2 Melting curves of the duplex between PNA-1 and its complementary 10-mer DNA (DNA-1) before the reduction with 1 mM TCEP (the solid line) and after that (the broken line). The absorbance at 260 nm was measured under these conditions: [PNA-1] = 3.0 μM, [DNA-1] = 3.0 μM, and [NaCl] = 100 mM in pH 7.0 HEPES buffer (5.0 mM).

[a)] The absorbance was normalized to facilitate the comparison.

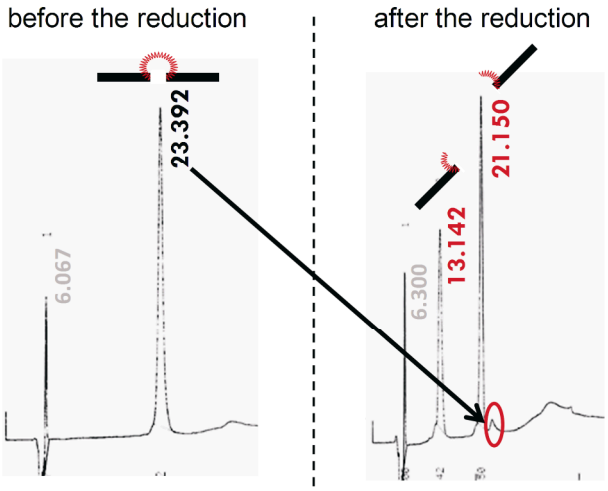

Fig. S3 HPLC assay on the reductive cleavage of the disulfide bond in PNA/DNA duplexes. Annealing conditions; [PNA-S1] = 3.33 μM, [DNA-1] = 5.0–10 μM in pH 7.0 HEPES buffer (5.56 mM). Then, 50 mM of TCEP was added to the sample solution to a final concentration of 5 mM and the mixture was placed on the ice bath throughout the reaction (Reaction conditions; [PNA-S1] = 3.0 μM, [DNA-1] = 4.5–9.0 μM and [TCEP] = 5.0 mM in pH 7.0 HEPES buffer (5.0 mM)). After the predetermined time (e.g. 45 min), the mixture was diluted by 5 times with water and then immediately injected into HPLC system. The efficiency of reductive cleavage did not depend on the ratio of PNA-S1 to complementary DNA (DNA-1) in the range employed here.