Parallel-Stranded Design of Peptide Nucleic Acids for Efficient Recognition of Double-Stranded DNA

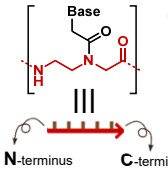
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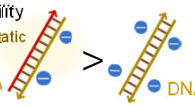
1. Introduction

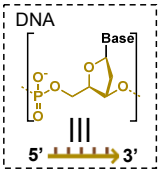
❑ Peptide Nucleic Acid (PNA) [1]



- electrostatically-neutral backbone
- stable duplex formation with DNA [2]

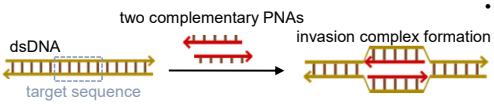
Thermal stability





Ref: [1] P. E. Nielsen *et al.*, *Science* **1991**, *254*, 1497. [2] M. Egholm *et al.*, Nature **1993**, *365*, 566.

❑ Double-duplex invasion by PNAs



PNAs can directly recognize target sequences in dsDNA.

This PNA invasion is expected to be a powerful tool for controlling dsDNA functions.

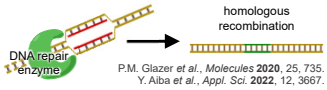
Applications

- Control of gene expression



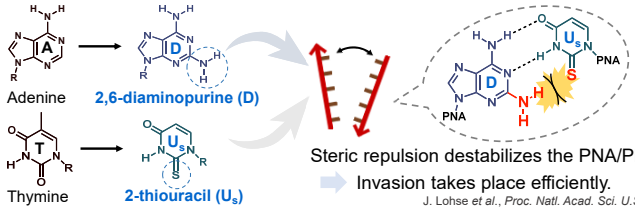
J. Lohse *et al.*, *Proc. Natl. Acad. Sci. USA.* **1999**, *96*, 11804..

- Genome editing



P.M. Glazer *et al.*, *Molecules* **2020**, *25*, 735.
Y. Aiba *et al.*, *Appl. Sci.* **2022**, *12*, 3667.

❑ Use of modified nucleobases (pseudo-complementary PNAs)



Steric repulsion destabilizes the PNA/PNA duplex.

Invasion takes place efficiently.

J. Lohse *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 11804.
M. Komiyama, Y. Aiba *et al.*, *Nat. Protoc.* **2008**, *3*, 646,

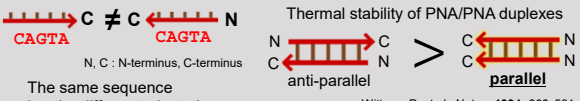
Problems

- Oligomer synthesis requires PNA monomers specially modified with unique nucleobases
  ➡ **Synthetic techniques are required**
- Not applicable to automatic synthesizers, limited to manual synthesis (Boc-protection)
  ➡ **Time-consuming and laborious**

Research aim: Development of a simple and accessible PNA invasion method (**using PNAs with natural nucleobases**)

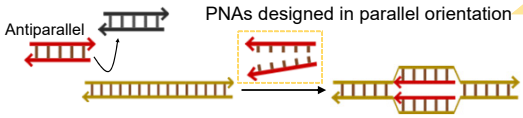
2. Strategy

Suppression of self-duplex formation by changing the orientation of nucleic acids



Wittung, P. *et al.*, *Nature* **1994**, *368*, 561.

▪ Approach in this study



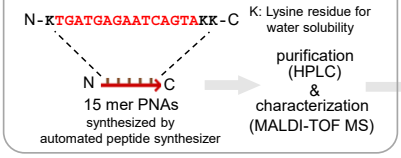
This approach does not require the modified nucleobases (pcPNAs).

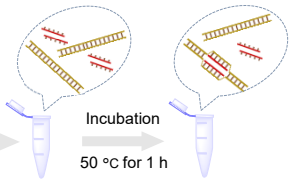
M. Shibata, **Y. Aiba** *et al.*, *RSC Chem. Biol.*, **2025**, *6*, 1566.

3. Results and Discussion

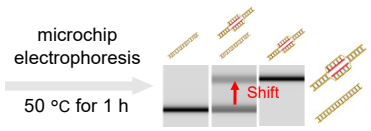
❑ Outline of invasion experiments

Synthesis of PNA oligomer
(Fmoc solid-phase peptide synthesis)



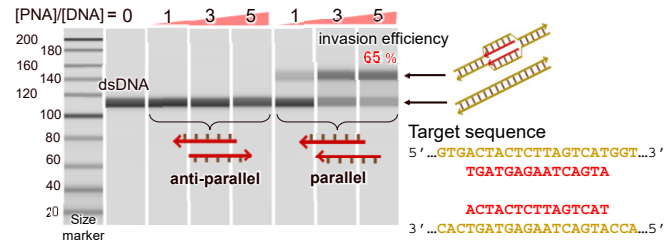


Conditions:
[DNA (119 bp)] = 100 nM
[each PNAs] = 100 - 500 nM
[HEPES (pH = 7.0)] = 5 mM



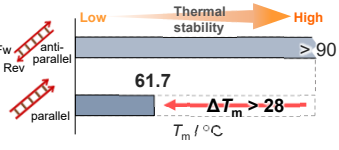
The formation of invasion complex was evaluated by EMSA.

❑ Evaluation of the invasion complex formation

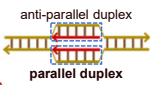


This result is the first reported instance of successful double-duplex invasion without the use of any modified nucleobases.

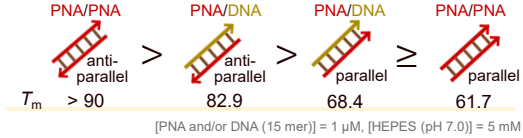
❑ Melting temperature analysis of PNA/PNA duplex



sequences of PNAs (15 bp)
Fw: ACTACTCTTAGTCAT
Rev: TGATGAGAATCAGTA



Thermal stability of PNA/PNA and PNA/DNA duplexes

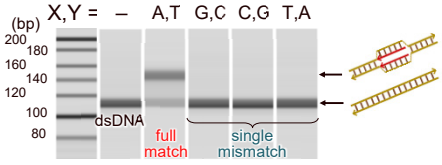


[PNA and/or DNA (15 mer)] = 1 μM, [HEPES (pH 7.0)] = 5 mM

The suppression of PNA/PNA duplex formation was effectively achieved as designed in parallel orientation.

❑ Mismatch sequence discrimination

Conditions: [DNA (119 bp)] = 100 nM, [each PNA] = 500 nM, [HEPES (pH = 7.0)] = 5 mM at 50 °C for 1 h.



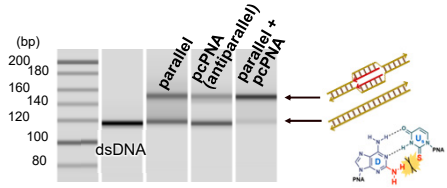
Single mismatch was introduced at the center of the target sequence.

5'...GTGACTACTCTAGTCATGGT...3'
TGATGACXATCAGTA
ACTACTCYAGTCAT
3'...CACTGATGACATCAGTACCA...5'

PNAs containing a single mismatch did not form an invasion complex.

➡ Parallel-stranded PNAs recognize dsDNA with high sequence selectivity.

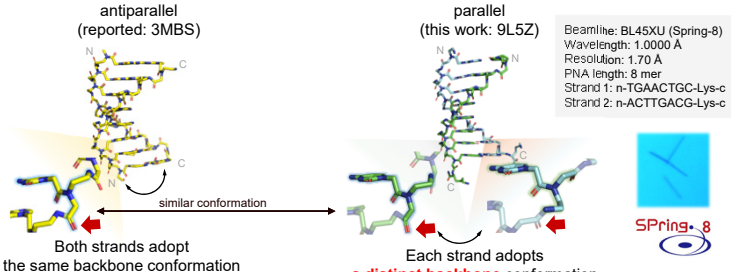
❑ Parallel design + pseudo-complementary nucleobases



Sequences of PNAs
parallel
C-TGATGAGAATCAGTA-N
C-ACTACTCTTAGTCAT-N
pcPNA (antiparallel)
N-TGATGD GAAUSCAGTA-C
C-ACTACUSCTTD GTCAT-N
parallel + pcPNA
C-TGATGD GAAUSCAGTA-N
C-ACTACUSCTTD GTCAT-N

Parallel invasion is effective even when combined with modified nucleobases.

❑ X-ray crystal structure of parallel PNA/PNA duplex



➡ The first crystal structure of parallel-stranded PNA/PNA was reported, revealing distinct structural differences compared to its antiparallel counterpart.

4. Summary

- ✓ Parallel-stranded PNAs form the invasion complex with dsDNA.
- ✓ High sequence specificity enables single mismatch discrimination of target sequences.
- ✓ The first crystal structure of a parallel-stranded PNA/PNA duplex was determined, revealing structural differences from its antiparallel counterpart.

M. Shibata, H. Sugimoto, M. Hibino, O. Shoji*, **Y. Aiba***, *RSC Chem. Biol.*, **2025**, *6*, 1566 (***outside back cover***).