

A Predictive Model for the Sequence-Dependent Fluorogenic Response of Forced-Intercalation Peptide Nucleic Acid (FIT-PNA)

Itamar Peled^a and Eylon Yavin^{a,*}

^aThe School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Hadassah Ein-Kerem, Jerusalem 91120, Israel.

*E-mail: eylony@ekmd.huji.ac.il; Fax: +972-2-6757574; [Tel: +972-2-6758692](tel:+972-2-6758692).

Tables of contents:

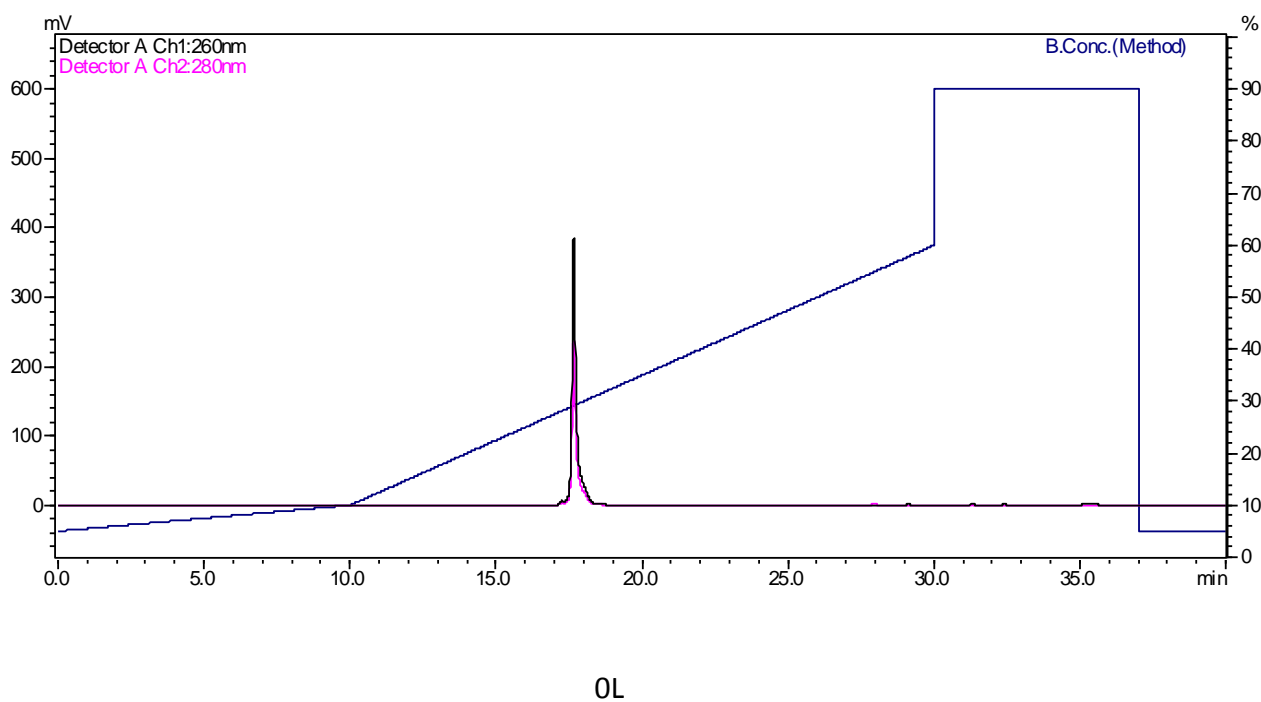
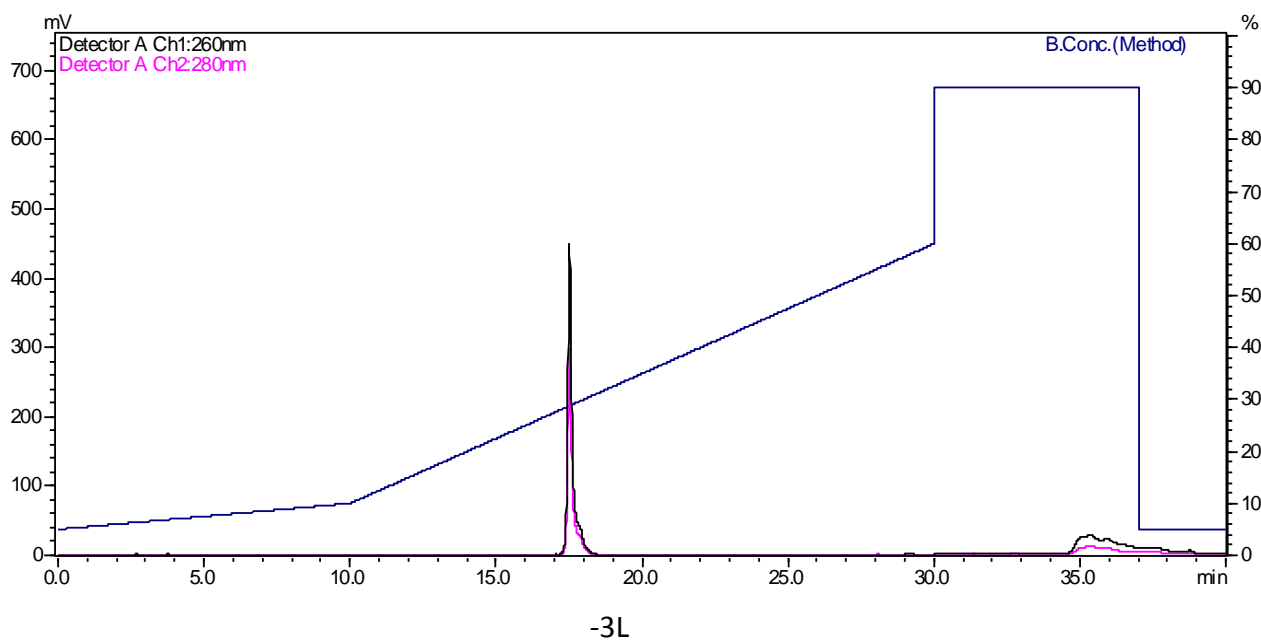
| | | |
|--|-----------|--------|
| HPLC chromatograms of pure FIT-PNAs | Figure S1 | S3-S5 |
| DNA sequences | Table S1 | S6 |
| DNA sequences for generating figures 2 and 3 | Table S2 | S7 |
| Calculation of ΔH NNI | | S8 |
| Data used to generate Figure 6 | Table S3 | S9-S11 |

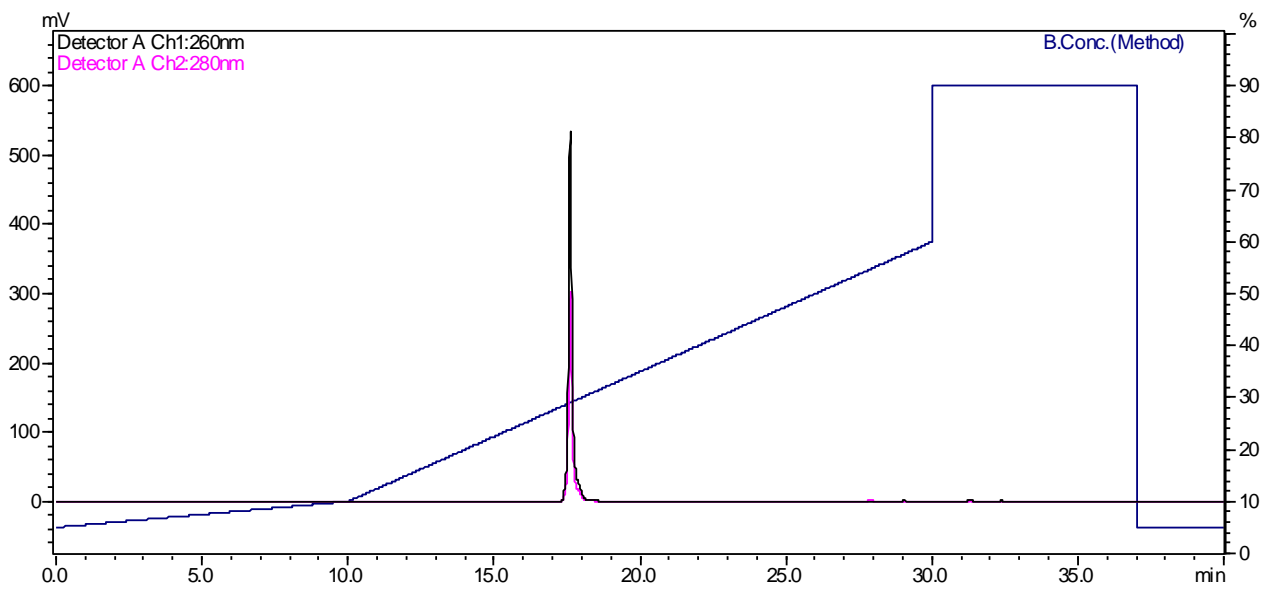
Figure S1. HPLC chromatograms of purified FIT-PNAs. Over 95% purity for all FIT-PNAs

RP-HPLC (Shimadzu LC2010), semi-preparative C18 reverse-phase column (Phenomenex, Jupiter 300 A) at a flow rate of 4 mL/min. Mobile phase: 0.1% TFA in H₂O (A) and acetonitrile (B).

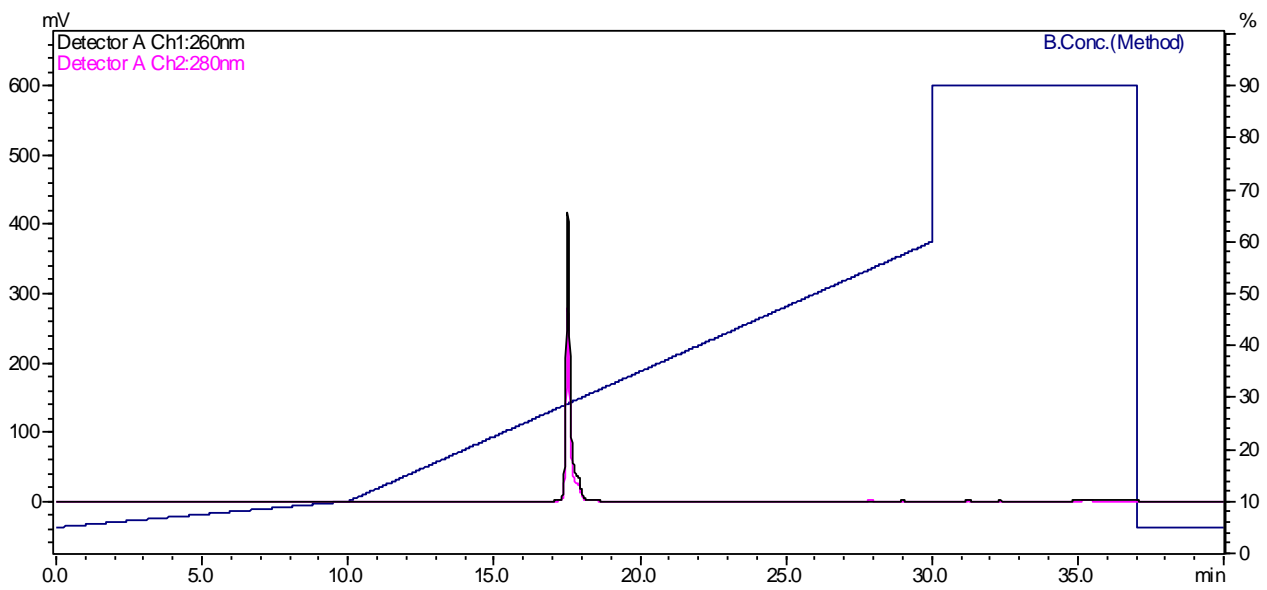
Gradient: 0-10 min: 90% A, 10% B. 10-30 min: linear gradient from 90% A-10%B to 40% A-60% B.

30-37 min: linear gradient from 40% A-60%B to 10% A-90% B. 37-40 min: 5% A-95% B.

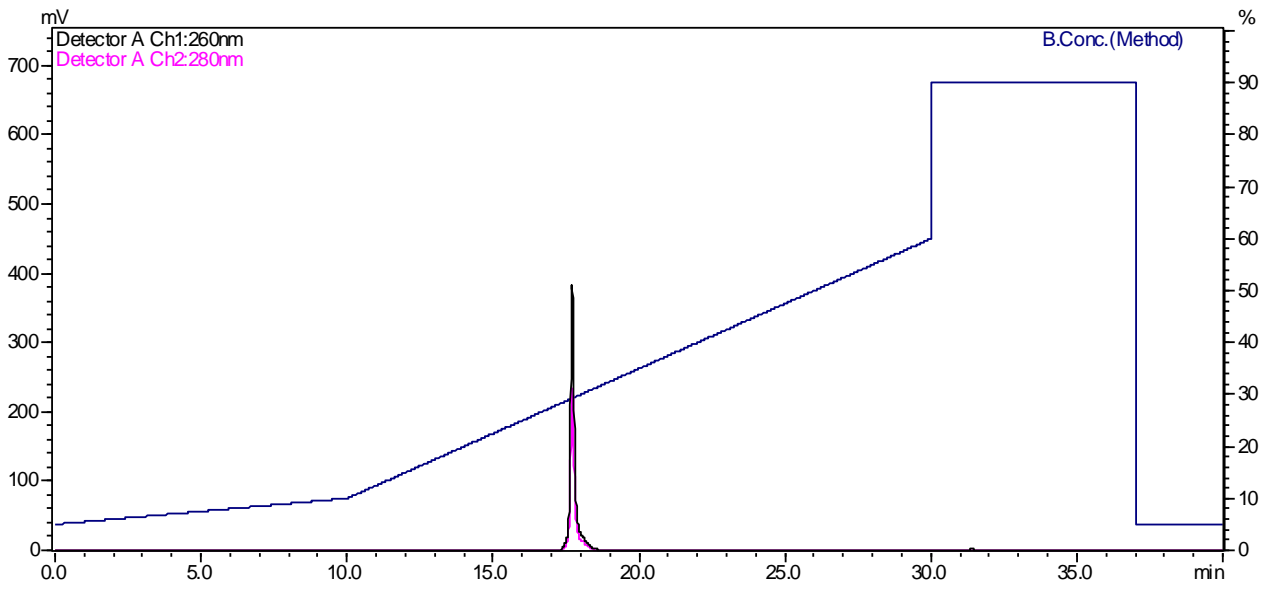




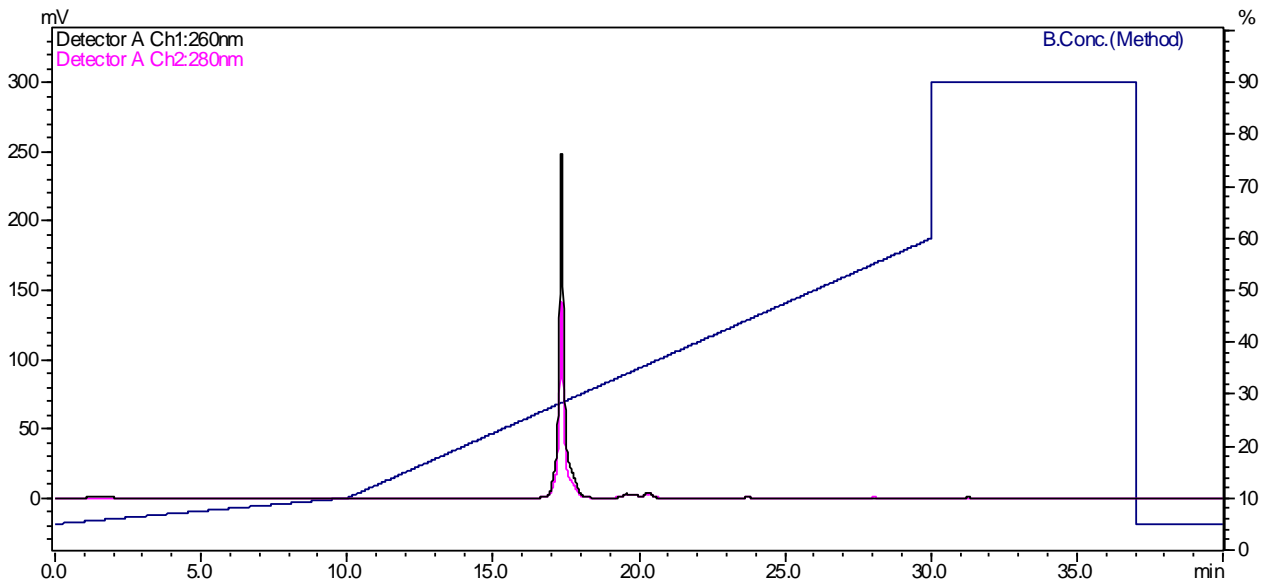
+3L



-3R



OR



+3R

DNA sequences:

A: ⁵CCCCTAGATCATCAGCTATGT**A**TGTTGCTTTTGATAATAAAAAT³
B: ⁵CCCCTAGATCATCAGCTATGT**G**TGTTGCTTTTGATAATAAAAAT³
C: ⁵CCCCTAGATCATCAGCTATGT**T**TGTTGCTTTTGATAATAAAAAT³
D: ⁵CCCCTAGATCATCAGCTATGT**C**TGTTGCTTTTGATAATAAAAAT³
E: ⁵CCCCTAGATCATCAGCTATGA**A**TGTTGCTTTTGATAATAAAAAT³
F: ⁵CCCCTAGATCATCAGCTATGC**A**TGTTGCTTTTGATAATAAAAAT³
G: ⁵CCCCTAGATCATCAGCTATGG**A**TGTTGCTTTTGATAATAAAAAT³
H: ⁵CCCCTAGATCATCAGCTAT**T**TATGTTGCTTTTGATAATAAAAAT³
I: ⁵CCCCTAGATCATCAGCTAT**A**T**A**TGTTGCTTTTGATAATAAAAAT³
J: ⁵CCCCTAGATCATCAGCTAT**C**T**A**TGTTGCTTTTGATAATAAAAAT³
K: ⁵CCCCTAGATCATCAGCT**A**AGT**A**TGTTGCTTTTGATAATAAAAAT³
L: ⁵CCCCTAGATCATCAGCT**G**T**G**T**A**TGTTGCTTTTGATAATAAAAAT³
M: ⁵CCCCTAGATCATCAGCA**A**TGT**A**TGTTGCTTTTGATAATAAAAAT³
N: ⁵CCCCTAGATCATCAGCTATGT**A**A**G**TGTTGCTTTTGATAATAAAAAT³
O: ⁵CCCCTAGATCATCAGCTATGT**A**C**G**TGTTGCTTTTGATAATAAAAAT³
P: ⁵CCCCTAGATCATCAGCTATGT**A**G**G**TGTTGCTTTTGATAATAAAAAT³
Q: ⁵CCCCTAGATCATCAGCTATGT**A**T**T**TGCTTTTGATAATAAAAAT³
R: ⁵CCCCTAGATCATCAGCTATGT**A**T**A**TGCTTTTGATAATAAAAAT³
S: ⁵CCCCTAGATCATCAGCTATGT**A**T**C**TGCTTTTGATAATAAAAAT³
T: ⁵CCCCTAGATCATCAGCTATGT**A**T**G**A**T**GCTTTTGATAATAAAAAT³
U: ⁵CCCCTAGATCATCAGCTATGT**A**T**G**T**A**GCTTTTGATAATAAAAAT³
V: ⁵CCCCTAGATCATCAGCTATGT**A**T**G**T**A**CTTTTGATAATAAAAAT³
W: ⁵CCCCTAGATCATCAGCTATGT**A**T**G**T**C**TTTTGATAATAAAAAT³

Table S1: A-W DNA sequences were hybridized to FIT-PNA's to create a 114 mismatched FIT-PNA:DNA duplexes ('A' corresponds to K13-C580Y and 'G' to K13-WT.)

Table S2: DNA sequences used to create data in Figures 2 and 3:

‘R’ PNA’s: ³TAGTCGATACAT**BC**AAACGAAAAC⁵
⁵CCCCTAGATCATCAGCTATGT**AT**GTTGCTTTTGATAATAAAAT³
 ‘L’ PNA’s: ³TAGTCGATAC**BT**ACAACGAAAAC⁵

DNA SEQUENCES:

A: ⁵CCCCTAGATCATCAGCTATGT**AT**GTTGCTTTTGATAATAAAAT³
B: ⁵CCCCTAGATCATCAGCTATGT**G**TGTTGCTTTTGATAATAAAAT³
C: ⁵CCCCTAGATCATCAGCTATGT**AA**TGTTGCTTTTGATAATAAAAT³
D: ⁵CCCCTAGATCATCAGCTAT**ATA**TGTTGCTTTTGATAATAAAAT³
E: ⁵CCCCTAGATCATCAGCT**AA**GT**AT**GTTGCTTTTGATAATAAAAT³
F: ⁵CCCCTAGATCATCAGCT**G**TGT**AT**GTTGCTTTTGATAATAAAAT³
G: ⁵CCCCTAGATCATCAGCA**AA**TGT**AT**GTTGCTTTTGATAATAAAAT³
H: ⁵CCCCTAGATCATCAGCTATGT**AA**GTTGCTTTTGATAATAAAAT³
I: ⁵CCCCTAGATCATCAGCTATGT**ATA**TGTTGCTTTTGATAATAAAAT³
J: ⁵CCCCTAGATCATCAGCTATGT**ATGA**TGTTGCTTTTGATAATAAAAT³
K: ⁵CCCCTAGATCATCAGCTATGT**ATGTA**GCTTTTGATAATAAAAT³
L: ⁵CCCCTAGATCATCAGCTATGT**ATGTTA**CTTTTGATAATAAAAT³

A-L DNA sequences are hybridized to FIT-PNA’s to create a duplex with a mismatch at a position with a defined distance and direction from BisQ. (‘A’ corresponds to K13-C580Y and ‘B’ to K13-WT.)

K13-C580Y SNP position nucleotide is marked in red, mismatched position nucleotide is highlighted in yellow.

For example: the **‘0L’:‘E’** PNA:DNA mismatched duplex is a ‘-2’ mismatch, since the mismatch position of this duplex is 2 bases from BisQ towards the DNA 5’ direction:

‘E’ DNA: ⁵CCCCTAGATCATCAGCT**AA**GT**AT**GTTGCTTTTGATAATAAAAT³
‘0L’ PNA: ³TCGAT**ACBT**ACAACGAAA⁵

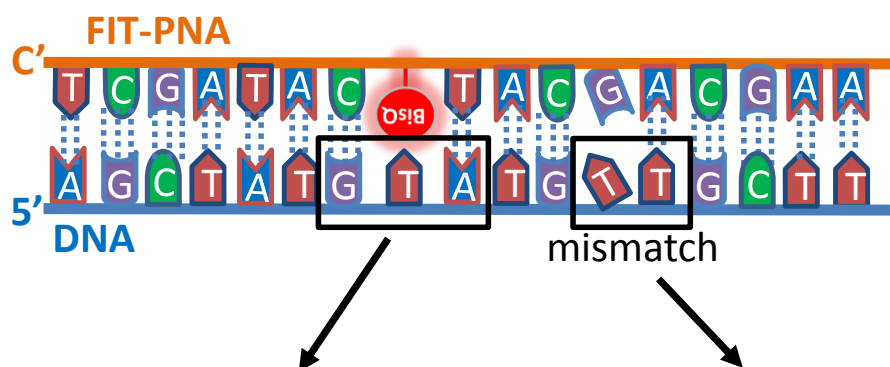
The same DNA sequence will create a ‘-4’ mismatch when hybridized with ‘0R’ FIT-PNA to create the **‘0R’:‘E’** PNA:DNA mismatched duplex, since now the mismatch position of the duplex is 4 bases from BisQ to the DNA 5’ direction:

‘E’ DNA: ⁵CCCCTAGATCATCAGCT**AA**GT**AT**GTTGCTTTTGATAATAAAAT³
‘0R’ PNA: ³TCGAT**ACATB**CAACGAAA⁵

Calculation of ΔH NNI

The predictive value for each duplex was calculated by summing the ΔH° (NNI) of the two NNI's in the nucleobases triad opposite BisQ and the NNI of the base stack 5' to 3' from the DNA mismatched base. The general equation is shown below:

$$\text{NNI factor} = \Delta H^\circ_{\text{NNI}} (\text{BisQ triad}) + \Delta H^\circ_{\text{NNI}} (\text{mismatch})$$



$$\Delta H^\circ_{\text{NNI}} (\text{BisQ triad}) = \Delta H^\circ (\text{GT}) + \Delta H^\circ (\text{TA})$$

$$\Delta H^\circ_{\text{NNI}} (\text{mismatch}) = \Delta H^\circ (\text{TT})$$

For a mismatch occurring adjacent to BisQ at the 5' end, one of the NNI of the triad opposite BisQ is considered un-stacked, and therefore calculated as zero.

For example: the '-3L':'U' PNA:DNA mismatched duplex shown below has a GT+TA triad NNI and an AG NNI 5' to 3' from the mismatched DNA nucleobase.



ΔH° (GT) = 6.5, ΔH° (TA) = 6.0 and ΔH° (AG) = 7.8.

Therefore the predicted value for the '-3L':'U' duplex is $\Delta H^\circ_{\text{total}} = 6.5+6+7.8=20.3$ (Table 1 shows ΔH values).

Table S3: Data used to generate the scatter plot (**Figure 6**) including statistical analysis and NNI calculations:

| 'L' N=3 results and outlier analysis | | | | | | | | | | | | | | | | | | | | |
|--|------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| | M | L | K | H | I | J | C | D | B | N | P | O | Q | R | S | T | U | W | V | |
| -3L | exp1 | 0.48 | 0.47 | 0.86 | 0.62 | 0.39 | 0.46 | 0.81 | 0.53 | 0.53 | 0.26 | 0.67 | 0.63 | 1.09 | 0.42 | 0.94 | 0.57 | 0.70 | 0.92 | 0.82 |
| | exp2 | 0.70 | 1.28 | 1.12 | 0.56 | 0.40 | 0.49 | 0.91 | 0.62 | 0.69 | 0.67 | 0.68 | 0.83 | 1.05 | 0.55 | 0.75 | 0.99 | 0.84 | 1.02 | 1.04 |
| | exp3 | 0.78 | 1.06 | 0.96 | 0.60 | 0.51 | 0.41 | 0.97 | 0.58 | 0.74 | 0.64 | 0.70 | 0.65 | 1.04 | 0.64 | 0.67 | 0.88 | 0.82 | 1.03 | 0.99 |
| 0L | exp1 | 0.58 | 0.72 | 0.69 | 0.41 | 0.50 | 0.40 | 0.78 | 0.54 | 0.75 | 0.26 | 0.63 | 0.82 | 1.17 | 0.47 | 1.04 | 0.68 | 0.55 | 0.66 | 0.49 |
| | exp2 | 0.84 | 1.22 | 0.97 | 0.51 | 0.47 | 0.53 | 0.92 | 0.62 | 0.77 | 0.65 | 0.84 | 1.15 | 1.07 | 0.60 | 0.78 | 0.94 | 0.80 | 0.92 | 0.94 |
| | exp3 | 0.63 | 0.98 | 0.75 | 0.55 | 0.50 | 0.50 | 1.03 | 0.65 | 0.80 | 0.55 | 0.57 | 0.71 | 1.11 | 0.67 | 0.77 | 0.77 | 0.67 | 0.78 | 0.76 |
| +3L | exp1 | 0.90 | 0.90 | 0.41 | 0.66 | 0.57 | 0.65 | 1.06 | 0.73 | 0.89 | 0.59 | 0.78 | 0.82 | 1.26 | 0.78 | 0.94 | 0.81 | 0.75 | 0.91 | 1.02 |
| | exp2 | 0.67 | 0.69 | 0.64 | 0.53 | 0.50 | 0.48 | 0.98 | 0.63 | 0.71 | 0.49 | 0.60 | 0.70 | 1.19 | 0.73 | 0.89 | 0.70 | 0.65 | 0.72 | 0.96 |
| | exp3 | 0.92 | 0.95 | 0.85 | 0.56 | 0.53 | 0.53 | 1.01 | 0.68 | 0.77 | 0.66 | 0.73 | 0.76 | 1.33 | 0.76 | 0.89 | 0.89 | 0.84 | 0.94 | 1.01 |
| | //If Avg | 0.72 | 0.92 | 0.81 | 0.56 | 0.48 | 0.49 | 0.94 | 0.62 | 0.74 | 0.53 | 0.69 | 0.79 | 1.15 | 0.62 | 0.87 | 0.80 | 0.73 | 0.88 | 0.89 |
| | SD | 0.15 | 0.26 | 0.21 | 0.07 | 0.06 | 0.08 | 0.10 | 0.06 | 0.10 | 0.16 | 0.09 | 0.15 | 0.10 | 0.13 | 0.13 | 0.14 | 0.10 | 0.13 | 0.18 |
| | MIN | 0.48 | 0.47 | 0.41 | 0.41 | 0.39 | 0.40 | 0.78 | 0.53 | 0.53 | 0.26 | 0.57 | 0.63 | 1.04 | 0.42 | 0.67 | 0.57 | 0.55 | 0.66 | 0.49 |
| | MAX | 0.92 | 1.28 | 1.12 | 0.66 | 0.57 | 0.65 | 1.06 | 0.73 | 0.89 | 0.67 | 0.84 | 1.15 | 1.33 | 0.78 | 1.04 | 0.99 | 0.84 | 1.03 | 1.04 |
| | AVG-MIN | 0.24 | 0.45 | 0.39 | 0.14 | 0.10 | 0.16 | 0.09 | 0.09 | 0.21 | 0.27 | 0.12 | 0.16 | 0.11 | 0.21 | 0.20 | 0.23 | 0.18 | 0.22 | 0.40 |
| | MAX-AVG | 0.20 | 0.36 | 0.32 | 0.10 | 0.09 | 0.16 | 0.12 | 0.11 | 0.15 | 0.14 | 0.15 | 0.36 | 0.18 | 0.16 | 0.16 | 0.19 | 0.11 | 0.15 | 0.15 |
| | Gmax | 1.33 | 1.38 | 1.50 | 1.43 | 1.47 | 1.86 | 1.25 | 1.75 | 1.57 | 0.83 | 1.75 | 2.32 | 1.83 | 1.23 | 1.28 | 1.39 | 1.09 | 1.19 | 0.84 |
| | Gmin | 1.64 | 1.72 | 1.87 | 2.05 | 1.64 | 1.15 | 1.66 | 1.40 | 2.16 | 1.65 | 1.37 | 1.03 | 1.05 | 1.65 | 1.58 | 1.71 | 1.83 | 1.70 | 2.27 |
| | Gcrit 0.05 | 2.11 | 2.11 | 2.11 | 2.11 | 2.11 | 1.94 | 2.11 | 2.11 | 2.11 | 2.11 | 2.11 | 2.11 | 2.11 | 2.11 | 1.94 | 2.11 | 2.11 | 2.11 | 2.11 |
| 'L' statistics after outlier (P<0.05, Grubbs test) removal | | | | | | | | | | | | | | | | | | | | |
| | //If Avg | 0.72 | 0.92 | 0.81 | 0.56 | 0.48 | 0.49 | 0.94 | 0.62 | 0.76 | 0.53 | 0.69 | 0.74 | 1.15 | 0.62 | 0.87 | 0.80 | 0.73 | 0.88 | 0.93 |
| | SD | 0.15 | 0.26 | 0.21 | 0.07 | 0.06 | 0.08 | 0.10 | 0.06 | 0.06 | 0.16 | 0.09 | 0.08 | 0.10 | 0.13 | 0.13 | 0.14 | 0.10 | 0.13 | 0.10 |

| 'L' | | | | | | | | | | | | | | | | | | | |
|------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| | M | L | K | H | I | J | C | D | B | N | P | O | Q | R | S | T | U | W | V |
| LNNI(BI+O) | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 | 15.6 | 12.1 | 12.3 | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 |
| Z-NNI(W/M) | 6 | 8.6 | 5.8 | 6.5 | 6.5 | 6.5 | 8.6 | 8.6 | 8.6 | 5.8 | 5.8 | 5.8 | 6.5 | 6.5 | 6.5 | 9.1 | 5.8 | 11.1 | 11.1 |
| NNI Fac | 18.5 | 21.1 | 18.3 | 19 | 19 | 19 | 24.2 | 20.7 | 20.9 | 18.3 | 18.3 | 18.3 | 19 | 19 | 19 | 21.6 | 18.3 | 23.6 | 23.6 |
| //If Avg | 0.72 | 0.92 | 0.81 | 0.56 | 0.48 | 0.49 | 0.94 | 0.62 | 0.76 | 0.53 | 0.69 | 0.74 | 1.15 | 0.62 | 0.87 | 0.80 | 0.73 | 0.88 | 0.93 |
| //If SD | 0.15 | 0.26 | 0.21 | 0.07 | 0.06 | 0.08 | 0.10 | 0.06 | 0.06 | 0.16 | 0.09 | 0.08 | 0.10 | 0.13 | 0.13 | 0.14 | 0.10 | 0.13 | 0.10 |

| 'R' N=3 results and outlier analysis | | | | | | | | | | | | | | | | | | | | |
|--|----------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| | M | L | K | H | I | J | E | G | F | C | D | B | Q | R | S | T | U | W | V | |
| -3R | exp1 | 0.63 | 0.84 | 0.94 | 0.91 | 0.77 | 0.84 | 0.54 | 0.76 | 0.89 | 0.98 | 0.62 | 0.49 | 0.82 | 0.75 | 0.98 | 0.74 | 0.56 | 1.24 | 0.95 |
| | exp2 | 0.62 | 0.94 | 1.06 | 0.73 | 0.74 | 0.86 | 0.68 | 1.20 | 0.83 | 0.73 | 0.50 | 0.84 | 0.84 | 0.77 | 0.94 | 0.97 | 0.81 | 1.34 | 1.21 |
| | exp3 | 0.68 | 1.02 | 1.06 | 0.95 | 0.85 | 0.68 | 0.65 | 0.73 | 0.86 | 0.76 | 0.51 | 0.88 | 0.88 | 0.82 | 0.88 | 0.88 | 0.78 | 1.35 | 1.17 |
| OR | exp1 | 0.55 | 0.90 | 0.89 | 0.56 | 0.49 | 0.92 | 0.68 | 0.83 | 0.62 | 0.61 | 0.48 | 0.72 | 0.60 | 0.75 | 0.84 | 0.53 | 0.53 | 1.09 | 1.00 |
| | exp2 | 0.74 | 0.96 | 0.91 | 0.76 | 0.75 | 0.88 | 0.70 | 1.11 | 0.71 | 0.67 | 0.50 | 0.77 | 0.77 | 0.64 | 0.92 | 0.76 | 0.76 | 1.25 | 1.10 |
| | exp3 | 0.79 | 1.10 | 0.99 | 0.79 | 0.75 | 0.88 | 0.75 | 0.92 | 0.71 | 0.68 | 0.46 | 0.78 | 0.58 | 0.71 | 1.33 | 0.76 | 1.31 | 1.31 | 1.17 |
| +3R | exp1 | 0.83 | 1.03 | 0.47 | 1.25 | 1.30 | 0.95 | 0.63 | 0.97 | 0.69 | 0.77 | 0.61 | 0.97 | 0.64 | 0.71 | 0.83 | 0.64 | 1.19 | 1.03 | 1.03 |
| | exp2 | 0.92 | 1.27 | 0.99 | 0.83 | 1.06 | 0.77 | 0.76 | 1.06 | 0.58 | 0.62 | 0.49 | 0.62 | 0.53 | 0.60 | 0.92 | 0.72 | 1.32 | 1.32 | 1.11 |
| | exp3 | 0.91 | 1.09 | 0.96 | 0.83 | 1.03 | 0.87 | 0.71 | 1.00 | 0.57 | 0.62 | 0.46 | 0.83 | 0.50 | 0.73 | 0.91 | 0.73 | 1.25 | 1.07 | 1.07 |
| | Wif Avg | 0.74 | 1.02 | 0.92 | 0.84 | 0.87 | 0.68 | 0.77 | 0.95 | 0.73 | 0.67 | 0.50 | 0.80 | 0.64 | 0.77 | 0.93 | 0.70 | 1.26 | 1.09 | 1.09 |
| | SD | 0.13 | 0.13 | 0.18 | 0.19 | 0.24 | 0.06 | 0.07 | 0.16 | 0.14 | 0.06 | 0.04 | 0.10 | 0.12 | 0.16 | 0.17 | 0.10 | 0.08 | 0.09 | 0.09 |
| | MIN | 0.55 | 0.84 | 0.47 | 0.56 | 0.49 | 0.77 | 0.54 | 0.72 | 0.57 | 0.61 | 0.46 | 0.62 | 0.50 | 0.60 | 0.74 | 0.53 | 0.53 | 1.09 | 0.95 |
| | MAX | 0.92 | 1.27 | 1.06 | 1.25 | 1.30 | 0.95 | 0.76 | 1.20 | 0.98 | 0.77 | 0.61 | 0.97 | 0.82 | 0.98 | 1.33 | 0.81 | 1.35 | 1.21 | 1.21 |
| | AVG-MIN | 0.19 | 0.17 | 0.45 | 0.28 | 0.37 | 0.10 | 0.14 | 0.24 | 0.16 | 0.07 | 0.04 | 0.18 | 0.14 | 0.17 | 0.19 | 0.17 | 0.17 | 0.14 | 0.14 |
| | MAX-AVG | 0.18 | 0.25 | 0.14 | 0.40 | 0.44 | 0.07 | 0.08 | 0.25 | 0.25 | 0.09 | 0.11 | 0.16 | 0.18 | 0.21 | 0.40 | 0.11 | 0.09 | 0.12 | 0.12 |
| | Gmax | 1.36 | 1.99 | 0.81 | 2.14 | 1.86 | 1.18 | 1.19 | 1.55 | 1.84 | 1.41 | 2.49 | 1.67 | 1.60 | 1.35 | 2.43 | 1.08 | 1.14 | 1.14 | 1.34 |
| | Gmin | 1.49 | 1.37 | 2.51 | 1.51 | 1.58 | 1.61 | 2.14 | 1.47 | 1.16 | 1.05 | 0.89 | 1.88 | 1.24 | 1.07 | 1.15 | 1.73 | 2.03 | 1.65 | 1.65 |
| | Grit0.05 | 2.11 | 2.11 | 2.11 | 2.11 | 2.11 | 2.11 | 2.11 | 2.11 | 2.11 | 2.11 | 2.11 | 2.11 | 2.11 | 2.11 | 2.11 | 2.11 | 2.11 | 2.11 | 2.11 |
| 'R' statistics after outlier (P<0.05, Grubbs test) removal | | | | | | | | | | | | | | | | | | | | |
| | Wif Avg | 0.74 | 1.02 | 0.97 | 0.80 | 0.86 | 0.87 | 0.68 | 0.77 | 0.95 | 0.73 | 0.67 | 0.48 | 0.80 | 0.64 | 0.77 | 0.93 | 0.70 | 1.26 | 1.09 |
| | SD | 0.13 | 0.13 | 0.06 | 0.12 | 0.24 | 0.06 | 0.04 | 0.06 | 0.16 | 0.14 | 0.06 | 0.02 | 0.10 | 0.12 | 0.16 | 0.07 | 0.10 | 0.08 | 0.09 |

| 'R' | | | | | | | | | | | | | | | | | | | |
|-------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| | M | L | K | H | I | J | E | G | F | C | D | B | Q | R | S | T | U | W | V |
| 1.NNI(BisQ) | 14.4 | 14.4 | 14.4 | 14.4 | 14.4 | 14.4 | 14.4 | 14.4 | 14.4 | 5.8 | 5.8 | 5.8 | 17.7 | 14.2 | 14.6 | 14.4 | 14.4 | 14.4 | 14.4 |
| 2.NNI(MM) | 6 | 8.6 | 5.8 | 6.5 | 6.5 | 6.5 | 6 | 6 | 6 | 8.6 | 8.6 | 8.6 | 6.5 | 6.5 | 6.5 | 9.1 | 5.8 | 11.1 | 11.1 |
| NNI Fac | 20.4 | 23 | 20.2 | 20.9 | 20.9 | 20.9 | 20.4 | 20.4 | 20.4 | 14.4 | 14.4 | 14.4 | 24.2 | 20.7 | 21.1 | 23.5 | 20.2 | 25.5 | 25.5 |
| Wif Avg | 0.74 | 1.02 | 0.97 | 0.80 | 0.86 | 0.87 | 0.68 | 0.77 | 0.95 | 0.73 | 0.67 | 0.48 | 0.80 | 0.64 | 0.77 | 0.93 | 0.70 | 1.26 | 1.09 |
| Wif SD | 0.13 | 0.13 | 0.06 | 0.12 | 0.24 | 0.06 | 0.04 | 0.06 | 0.16 | 0.14 | 0.06 | 0.02 | 0.10 | 0.12 | 0.16 | 0.07 | 0.10 | 0.08 | 0.09 |

Table S3 shows the data analysis and NNI factor calculations for generating Figure 6 in manuscript. The two tables present the I_f values obtained for each PNA:DNA duplex (N=3 repetitions). Letters (in orange) notate the DNA sequences as given in Table S1 and -3\0\+3 (in light blue) notate the FIT-PNAs. Each data point corresponds to an I_f value. Next, the average I_f and standard deviation (SD) of each FIT-PNA frame (-3\0\+3) for N=3 is shown. Grubs test statistics to evaluate outliers in the data is shown next in the table, performed over the nine values of each averaged group. Outliers of P=0.05 (in red) were excluded and I_f and standard deviation was then recalculated. The NNI factor calculation table is shown beneath each data table; for each DNA sequence the BisQ triad and mismatch NNI values are shown followed by the total NNI factor as their sum. I_f and its standard deviation are shown again for convenience.