# Using PNA Oligomers for RNA Visualization and Function Inhibition

Rutu J. Jagtap and Andres Romero, M.S. in Biotechnology Management & Entrepreneurship
Irina E. Catrina, Ph.D., Yeshiva College, Dept. of Chemistry and Biochemistry

**FACULTY MENTORS: Rana Khan, Ph.D.**

Katz
Katz School of Science and Health

## Introduction

Messenger RNA (mRNA) translation is a critical cellular process, and its precise temporal and spatial regulation has far-reaching implications in molecular biology and medicine.

Peptide Nucleic Acids (PNA) are synthetic nucleic acid analogs with a unique peptide-like backbone making them resistant to enzymatic degradation. Due to their high binding specificity, PNAs form stable complexes with RNA and DNA, providing a novel strategy for translational inhibition and RNA detection and visualization.

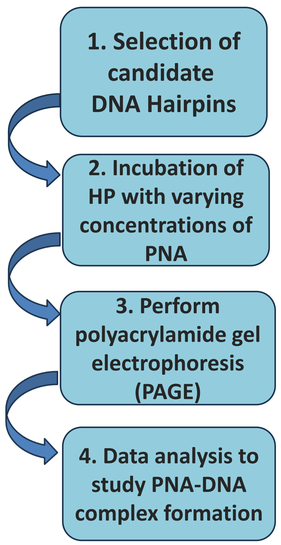
It is critical to understand the interaction dynamics between PNAs and nucleic acids. Moreover, the potential of PNAs to be utilized as *in vivo* imaging tools to study RNA trafficking dynamics during organism developmental processes was explored.

Understanding these interactions will enable us to develop new strategies for controlling gene expression and visualizing RNA transport and localization in live cells.

**Aims:** This study set out to identify the molecular principles governing PNA-double stranded DNA (dsDNA) complex formation, assess the feasibility of performing PNA-mediated translation inhibition, and evaluate the use of PNA oligomers in RNA detection.

By investigating the interactions between PNA oligomers and DNA hairpin (HP) models derived from the sequence of *oskar* mRNA, which is essential for posterior axis determination in *Drosophila* embryos, this research will provide insights into the PNA's potential for translational inhibition and live-cell imaging during egg chamber development.

## Methods



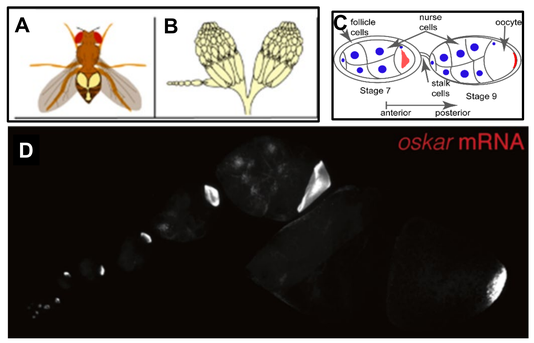




**Fig. 1:** Female fruit fly **(A),** contains a pair of ovaries **(B),** composed of ovarioles, which are made of egg chambers at various stages of development **(C, D),** with *oskar* mRNA presenting a posterior localization beginning in stage 9 **(C, D)** (1).

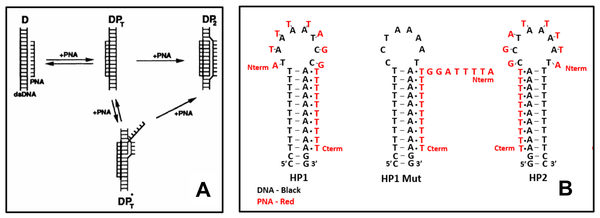



**Fig. 2: (A)** Proposed model for DNA HP/PNA complex formation (2). **(B)** Structure of complexes proposed to form between the corresponding HPs (black) and PNA (red) showing Watson-Crick (-) and Hoogsteen (•) base pairing.

## Results and Discussion

- The interaction between pre-folded DNA HPs (0.5 μM) and varying concentrations of PNA oligomer of up to 2 μM was studied in buffer solution containing 100 mM NaCl and incubated at room temperature for 1 hour. The HPs were folded by heating at 95°C for 1 min followed by slow cooling to 25°C and storing on ice. Reactions were resolved using native PAGE and bands were visualized by SYBR Gold staining.

- DNA HP/PNA multimer complexes formed (Fig 2A, duplex invasion) with the rate constant $k_{ps}$ as the relevant parameter since the corresponding kinetic products were formed in presence of excess PNA and equilibrium was not reached. Therefore, DNA HP/PNA complex formation was analyzed for pseudo-first-order kinetics.

- The time course of binding was assessed by plotting $-ln(D/D_o)$ versus time, where $D_o$ was the total DNA HP concentration and $D$ was the free DNA HP. The linearity of these plots (Fig. 3B) confirmed the pseudo-first-order behavior, with $k_{ps}$ as the pseudo-first-order rate constant. To assess exponential relationship of $k_{ps}$ with PNA concentration ($k_{ps} \sim P^{\gamma}$), the slope $\gamma$ was calculated from the corresponding plots of $ln[-ln(D/D_o)]$ versus $ln([PNA]/[HP])$ (Fig. 3B).

- Among the tested hairpins, HP1, HP1 MUT and HP2, demonstrated a more efficient HP duplex invasion, which was evident from the pronounced gel shifts and steeper slopes (Fig. 4). While statistical analysis using pairwise Tukey comparisons did not find significant differences between these slopes, larger standard errors were obtained for four of six total HPs, and only three data points were used to calculate each slope. Additional experiments are underway to improve results by adding more data points for PNA concentration and improving pipetting of small volumes and gel loading techniques.

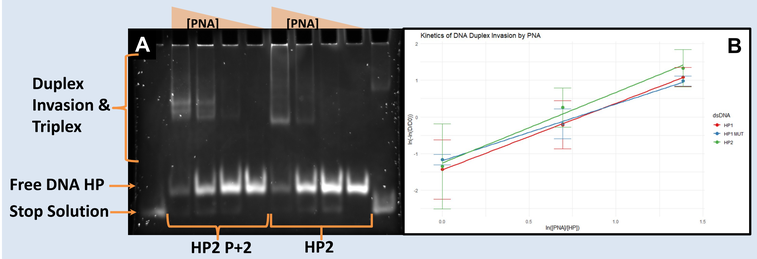



**Fig. 3: (A)** Example of native PAGE result for the indicated DNA HPs stained with SYBR Gold. Image acquired with a BioRad ChemiDoc instrument. Individual free DNA HP bands were analyzed using the Gel Analysis plugin of Fiji/Image J **(B)** Example of plot used to determine the slope (γ) for the indicated HPs.

| A dsDNA (# replicates) | Slope & std error |
|---|---|
| HP1 (7) | 1.816 ± 0.035 |
| HP1 MUT (2) | 1.544 ± 0.08 |
| HP1 P+2 (4) | 1.668 ± 0.024 |
| HP1 P+6 (4) | 2.007 ± 0.168 |
| HP2 (4) | 1.926 ± 0.223 |
| HP2 P+2 (5) | 1.148 ± 0.153 |
| HP2 P+7 (6) | 1.66 ± 0.126 |

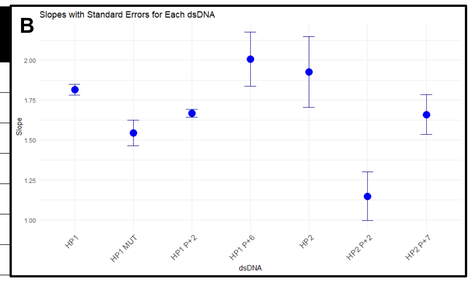



**Fig. 4: (A)** Slope (γ) values for the indicated DNA HPs calculated using a custom R script. Sequence variations for HP1 and HP2 are indicated as "P+n", with n as the number of nucleotides that can form a duplex with the loop of each HP immediately adjacent to the predicted Hoogsteen base pairs. **(B)** Graphical representation of slopes (γ) for indicated HPs.

## Conclusions

- This study demonstrated that PNA oligomers can efficiently invade A:T DNA hairpins in conditions of reduced PNA excess (4 vs. 200-fold), and at a salt concentration closer to physiological conditions (100 vs. 10 mM NaCl) then previously reported to be required for invasion of long double stranded DNAs (2). DNA duplex invasion by PNA oligomers followed pseudo-first-order kinetics, with similar, slightly smaller, slope values as long dsDNA.
- Among the hairpins studied, HP1, HP1 MUT and HP2, showed a greater efficiency in forming complexes with PNA. Although statistical analysis revealed no significant differences between hairpins, further experiments are planned to increase sample size and reproducibility.
- Our data suggest that designing PNAs capable to form triple and double strands may facilitate DNA duplex invasion for hairpins and can be extended to RNA targets for detection and visualization, as well as function impairment.

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