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Development of peptide nucleic acids-based molecules targeting non-canonical nucleic acids structures

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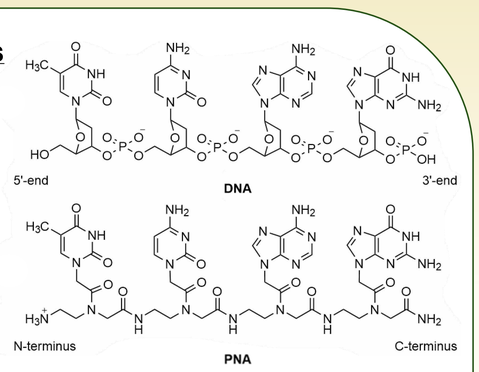
Introduction

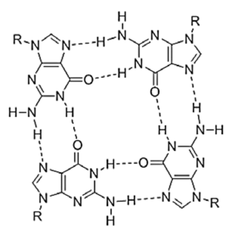
Peptide nucleic acids

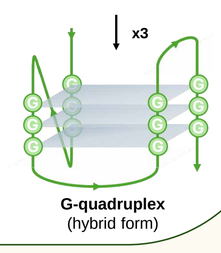
- Nucleobases linked to an **achiral**, **uncharged**, **flexible** polyamide backbone (generally N-2-aminoethylglycine units) through methylene carboxylic linkers.
- High **affinity** towards nucleic acids sequences.
- High PNA-nucleic acids **complex stability**.
- **Watson-Crick** and **Hoogsteen** base pairing.
- **Stability** towards nucleases and proteases.[1]



G-quadruplex (G4)

- Non-canonical nucleic acids structures abundant in guanine-rich sequences.
- Stacked guanine-based tetrads stabilized by Hoogsteen-hydrogen bonds.
- G-tracts arranged in different topologies: parallel, anti-parallel, hybrid.
- Abundant in proto-oncogenic promoters and in telomeric sequences, being deeply correlated with crucial cellular processes.
- Telomeric G4-stabilizing ligands are candidates for cancer therapy, despite challenges in **selectivity** and **specificity**.[2,3]





Synergize the base-pairing selectivity of PNA molecules with the capability of G4-ligands to stabilize G4 structures

Need for structural information about G4 PNA-DNA adducts

Design and synthesis

Design:

DNA Tel23 sequence:	5'- **TAGGGTTAGGGTTAGGGTTAGGG** -3'	**Homologous PNAs**
PNA1 sequence:	H- **TTAGGGTTAGGG** -NH_2	able to form stable PNA-DNA G4 adducts.[4]
PNA2 sequence:	H- **TAGGGTTAGGGT** -NH_2	
PNA3 sequence:	H- **GGGTTAGGGT** -Lys-Lys-NH_2	

DNA Tel23 sequence:	5'- **TAGGGTTAGGGTTAGGGTTAGGG** -3'	**Hybrid PNA**
PNA4 sequence:	H_2N-Lys-**ATCCCAATGGG** –H	hybrid complex by forming both a double helix and a G-quadruplex

Solid Phase Synthesis

Fmoc/t-Bu orthogonal protection strategy

PNA1, **PNA2**

- Resin: TentaGel® S RAM (0.23 mmol/g)
- Deprotection: 20% piperidine in DMF 5'+10'
- PNA monomers coupling: PNA monomer (4 eq), PyOxim (4 eq), DIPEA (8 eq) for 30' at room temperature. Double coupling for 1st, 10th, 11th, 12th residues.
- Capping: after each coupling step, 10% Ac_2O in DMF

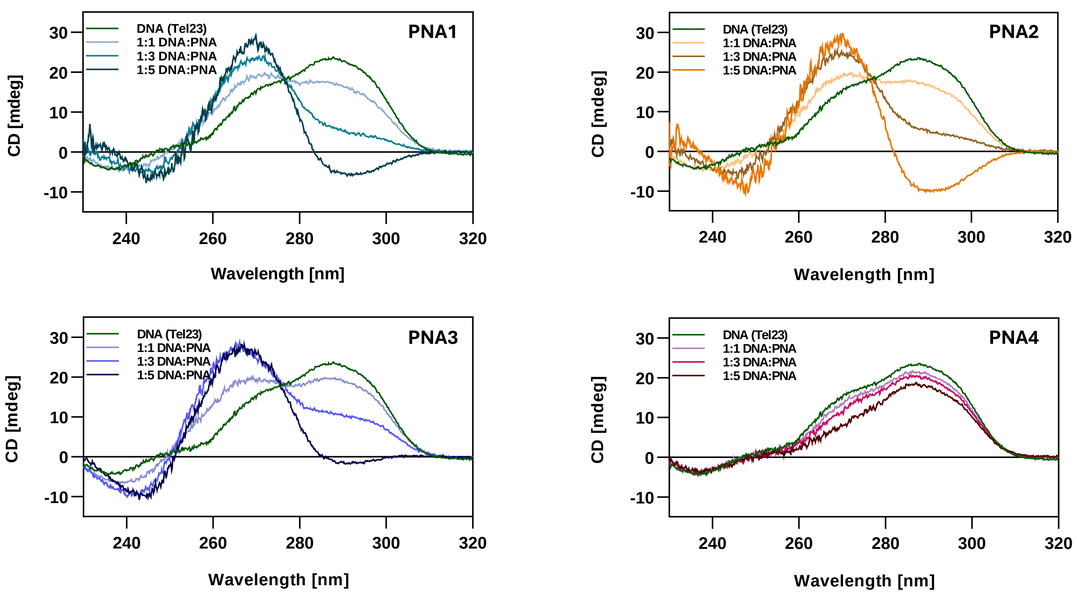
PNA3, PNA4

- Resin: TentaGel® R RAM (0.20 mmol/g)
- Deprotection: 20% piperidine in DMF 5'+10'
- Lys-coupling: Lys (5 eq), HBTU (5 eq), DIPEA (10 eq) for 30' at room temperature.
- PNA monomers coupling: PNA monomer (6 eq), PyOxim (6 eq), DIPEA (12 eq) for 40 min at room temperature.

Conformational and stability studies

Circular dichroism analysis

Samples with 4.7 μM DNA in the absence and presence of PNA were prepared to per form CD analyses at 20°C. Spectra were recorded in 80 mM KH_2PO_4 (pH 7.0). PNA1 and PNA2 were solubilized in DMSO (20 mM PNA).



DNA Tel23 → Hybrid G-quadruplex shape

PNA1, PNA2, PNA3 → 1:5 DNA:PNA parallel G-quadruplex shape

PNA4 → The decrease in signal around 270 nm, coupled with the absence of a minimum at 260 nm, might suggest the presence of an antiparallel G-quadruplex whose signal is overlapping with a duplex structure signal.

Melting temperatures

were determined using circular dichroism spectroscopy. Temperature gradient of 1°C from 20°C to 90°C. Heating rate of 0.2 °C/min, data pitch of 0.1 nm. For PNA1, PNA2, and PNA3, CD spectra were recorded from 266 nm to 266.5 nm, for DNA and PNA4 measurements were taken between 290 nm and 289.5 nm. DNA:PNA 1:5.

	T_m (°C)	ΔT_m (°C)
DNA	63.9 ± 0.1	-
PNA1	71.7 ± 0.2	+7,8
PNA2	70.7 ± 0.3	+6.8
PNA3	68.8 ± 0.2	+4,9
PNA4	65.5 ± 0.1	+1,6

CD analysis and melting temperatures suggest an interaction between DNA and PNAs that determines in all cases a conformational change of the Tel23 G-quadruplex and an increase in stability.

In silico studies

In silico studies (Maestro - Schroedinger suite) for the DNA-PNA complexes

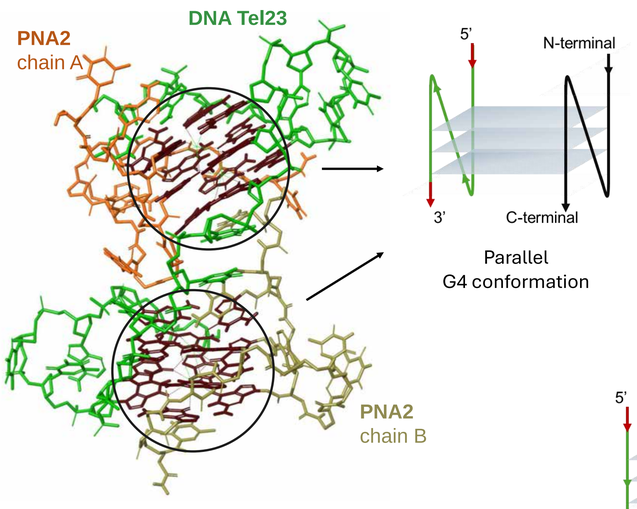
Construction

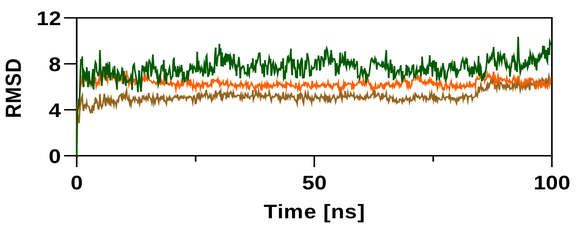
- Templates used for the homologous complexes:
  Bimolecular parallel-stranded human telomeric quadruplex in complex (PDB: 3CE5)
  PNA template: gamma-methylated PNA duplex (PDB: 2KVJ)
- Complex Features:
  Two bimolecular parallel-stranded DNA-PNA antiparallel G-quadruplexes (assuming the same topology that characterized the 3CE5 complex for all the G4s and the PNA N-terminus, aligns with DNA 5' -terminus)

- Templates used for the Hybrid complex:
  Biomolecular telomeric G4 structure: (3+1) assembly of three human telomeric DNA repeats into an asymmetrical dimeric G-quadruplex (PDB: 2AQY)
  PNA template: gamma-methylated PNA duplex (PDB: 2KVJ)
  DNA duplex template: CK14 DNA duplex (PDB: 1K8J)
- Complex Features:
  DNA-PNA double helix + (3+1) DNA-PNA antiparallel G-quadruplex (assuming the same topology that characterized the 2AQY complex and the PNA N-terminus, aligns with DNA 3' -terminus)

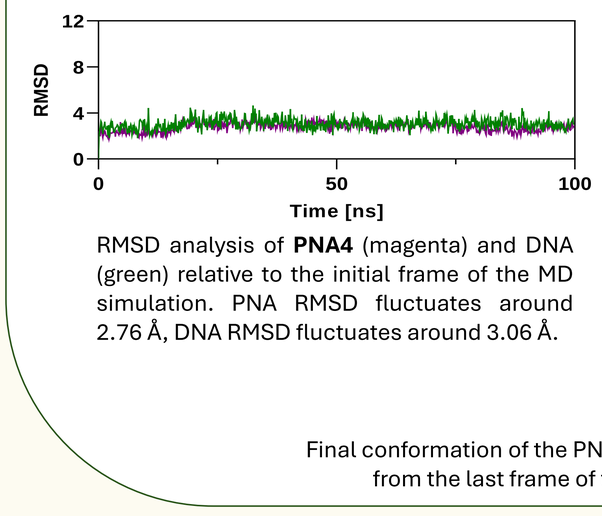
Molecular dynamics (MD) study:

Force Field: OPLS4
Time 100ns
Essemble class: NPT
Temperature: 300K
Pressure : 1.01325 bar

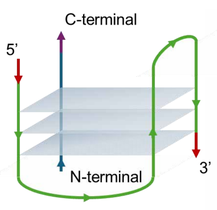


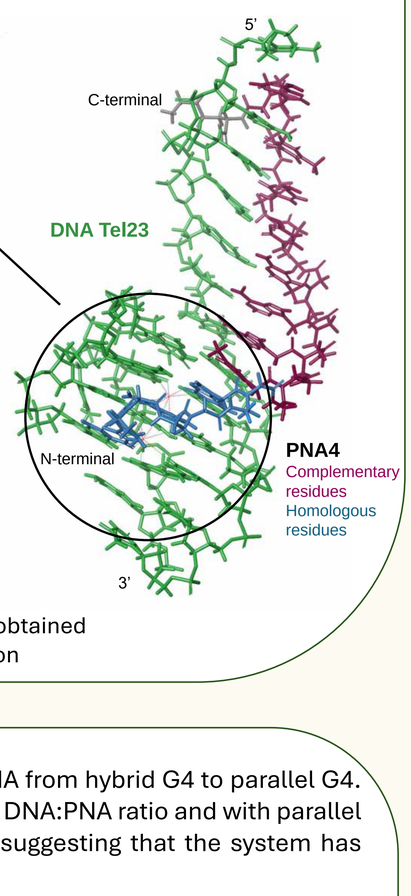


RMSD analysis of **PNA2** (orange, chain A, and ochre, chain B) and DNA (green) relative to the initial frame of the MD simulation. PNA RMSD fluctuates around 6.31 Å (chain A) and 5.20 Å (chain B), DNA RMSD fluctuates around 7.66 Å



RMSD analysis of **PNA4** (magenta) and DNA (green) relative to the initial frame of the MD simulation. PNA RMSD fluctuates around 2.76 Å, DNA RMSD fluctuates around 3.06 Å.





Final conformation of the PNA and DNA complexes obtained from the last frame of the 100 ns MD simulation

Conclusions and perspectives

- PNA1, PNA2, PNA3 determine a conformational change of Tel23 DNA from hybrid G4 to parallel G4. MD studies performed on PNA1 and PNA2 with Tel23 DNA with a 1:2 DNA:PNA ratio and with parallel G4 conformation returned RMSD values that appear to converge, suggesting that the system has reached a stable structural equilibrium during the simulation.
- Molecular dynamics studies performed on PNA4 and Tel23 returned convergent RMSD values suggesting that the system has reached a stable structural equilibrium during the simulation. CD studies on PNA4 and Tel23 DNA showed a conformational change of Tel23 DNA from hybrid G4 to possibly antiparallel G4 with additional formation of a double helix as observed in MD studies.
- All DNA-PNA complexes in 1:5 ratio showed higher melting temperatures than the DNA-DNA adduct suggesting a greater stability of the complexes.
- **Mass Spectrometry Analysis**: Perform precise mass spectrometry studies to determine the molecularity of the PNA-DNA complexes.
- **NMR and X-ray Crystallography**: Utilize nuclear magnetic resonance spectroscopy and X-ray crystallography to elucidate the conformations of the complexes and characterize the interactions crucial for complex formation.
- **Molecular Dynamics Simulations**: Conduct MD simulations to guide the structural modifications of hybrid and homologous PNAs, including amino acid conjugation, aimed at identifying molecules with enhanced interaction properties.
- **Ligand-PNA Conjugation**: Explore the design of ligand-PNA conjugated molecules to achieve selective and stable interactions with quadruplex DNA.

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