Flanking duplex can be a locking mechanism of structural polymorphism of AS1411 aptamer maintaining their properties?

André Miranda [1], Tiago Santos [1], Eric Largy [2], Carla Cruz [1] *

[1] CICS-UBI – Centro de Investigação em Ciências da Saúde, Universidade da Beira Interior, Covilhã, Portugal;
[2] Laboratoire Acides Nucléiques: Régulations Naturelle et Artificielle, Université de Bordeaux, INSERM & CNRS, (ARNA, U1212, UMR5320), IECB, 2 rue Robert Escarpit, 33607 Pessac, France;
**Email:* carlacruz@fcsaude.ubi.pt | andre.miranda@ubi.pt

Introduction

Aptamers

Recognize their targets with high affinity

K_D of pM to µM

AS1411
G-quadruplex (G4) aptamer

8 possible topologies
High polymorphic degree
Limits structural characterization

Binds to Nucleolin protein

Overexpressed in surface of Cancer Cells

Allow the application to cancer therapy and diagnosis

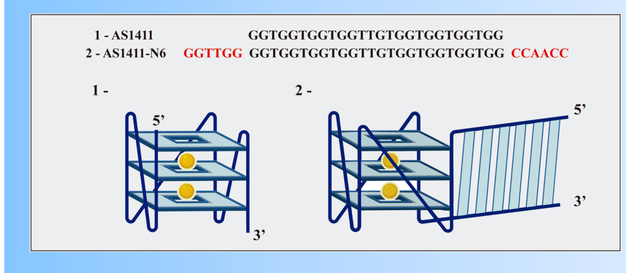
AIM:

Modify AS1411 to reduce structural polymorphism

Introduction of flanking duplex to retain a single topology

Methodology

Aptamer design



Effect of salt in G-Quadruplex

- Circular Dichroism (CD)
- UV-Vis Spectroscopy
- Nuclear Magnetic Resonance (NMR)

Evaluation of G4-ligands and Nucleolin interaction

Acridine | Pyridines | Phenanthroline | Porporphyrin

- FRET-melting Assay
- Surface Plasmon Resonance (SPR)
- PAGE

Results

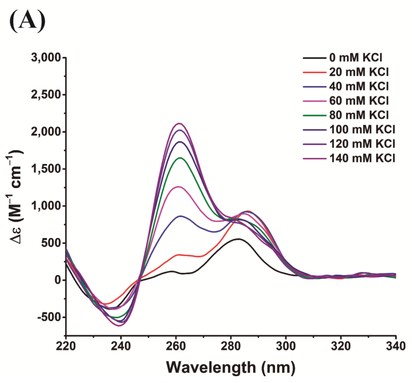


Fig. 1 – CD titration of 10 µM AS1411-N6 (A) and AS1411 (B) solutions containing K^+ (20-140 nM range).

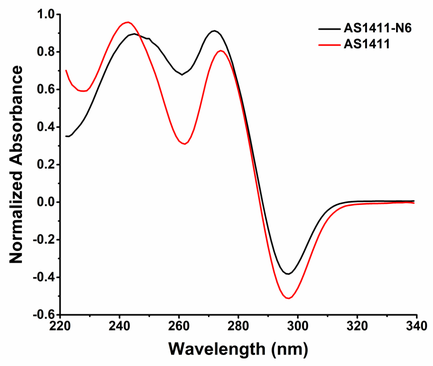


Fig. 2 –TDS spectra of AS1411-N6 and AS1411, performed in 10 mM of lithium cacodylate supplemented with 140 mM KCl. The graph was obtained by subtracting the spectrum at 25 °C from the spectrum at 90 °C, and then normalized relative to the maximum absorbance.

Table 1. Topology characterization of AS1411 and AS1411-N6 according to reference TDS values

	ΔA240/ ΔA295	ΔA255/ ΔA295	ΔA275/ ΔA295	Topology
AS1411	1.89	1.07	1.62	Antiparallel
AS1411-N6	2.27	2.12	2.34	Mixed

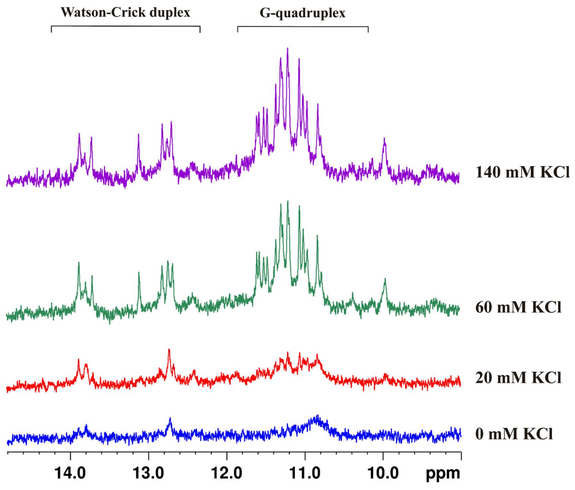


Fig. 3 – Study of KCl effect in the secondary structure of AS1411-N6 by ^{1}H NMR.

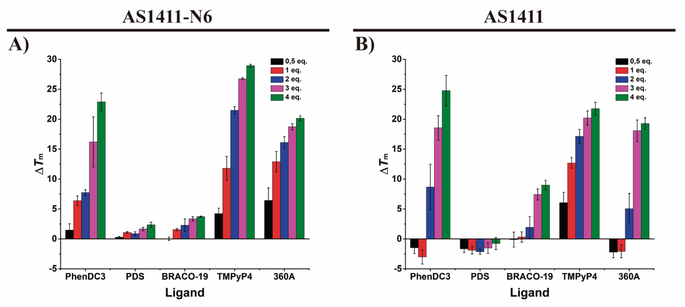


Fig. 4 – Thermal stabilization of aptamers induced by ligands obtained by FRET-melting experiments.

Table 2: KD (M) constant values of AS1411-N6 and AS1411 measured by SPR biosensor.

	PhenDC3	360A	TMPyP4
AS1411-N6	2.27×10^{-7}	3.82×10^{-8}	2.64×10^{-7}
AS1411	3.44×10^{-7}	4.34×10^{-8}	1.15×10^{-7}

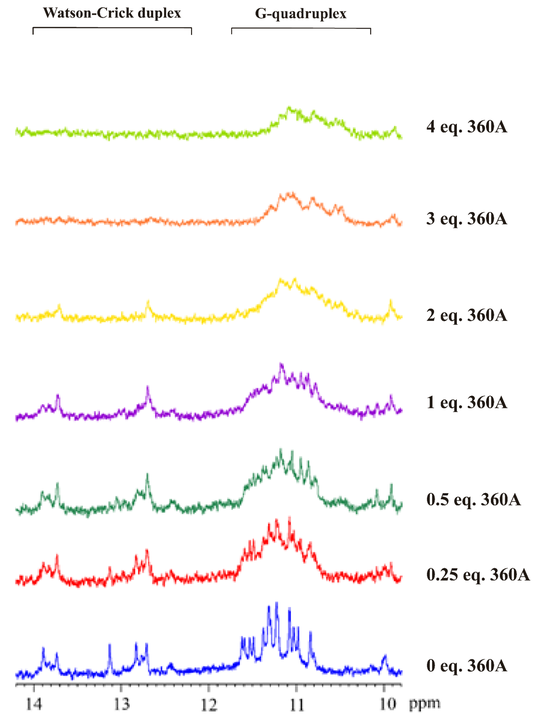


Fig. 5 – Imino region of AS1411-N6 upon 360A ligand titration evidencing disruption of duplex portion.

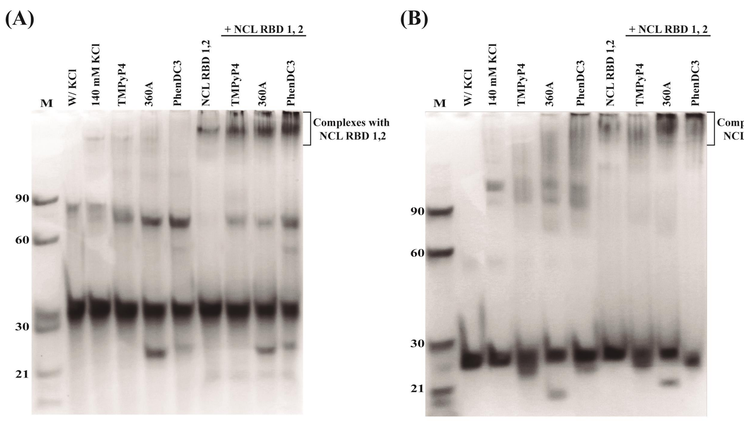


Fig. 6 – Polyacrylamide gel electrophoresis under native conditions of AS1411-N6 (A) and AS1411 (B)

Conclusions

- The results suggested a decrease in the structural polymorphism of the G4-forming aptamer AS1411-N6 compared to the original aptamer;
- Secondary structure of AS1411-N6 forms a duplex/G4 hybrid structure in the presence of K^+;
- PhenDC3, 360A, and TMPyP4 on both oligonucleotides showed promising stabilizer effects and SPR measurements showed that 360A is the most prominent affinity ligand;
- AS1411-N6 upon 360A NMR titration revealed a poorly resolved imino proton region, suggesting a less-defined binding of the ligand to AS1411-N6 and/or aptamer dimerization;

Acknowledgements
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