Higher order structures of oligonucleotides investigated by ion mobility mass spectrometry, and collision induced unfolding

Zeina Hajjar, Cédric Delvaux, Maxime Benonit, Christopher Kune, Gauthier Eppe, Edwin De Pauw and Johann Far

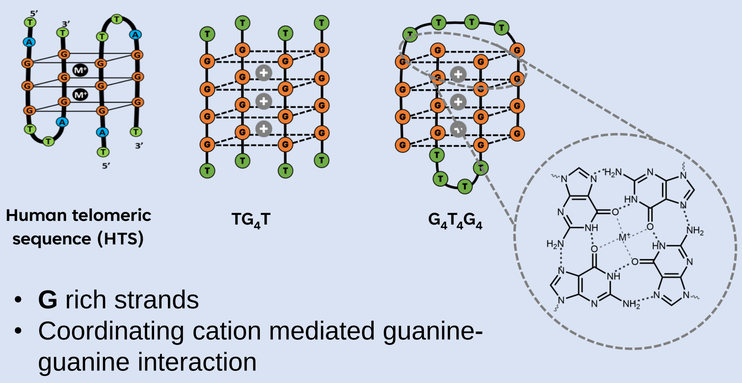
Mass Spectrometry Laboratory, MolSys Research Unit, Quartier Agora, University of Liège, Allée du Six Août 11, B-4000 Liège, Belgium

Introduction

Oligonucleotide(ON)-based therapeutics have grown significantly in the recent years. **ON** can adopt diverse conformations known as **higher order structures** (HOS) such as double helices or G-quadruplexes. These structural changes impact several physicochemical properties. As the **characterization** of these HOS is important notably for biological and chemical applications, innovative methods are needed to resolve these structures under different experimental conditions. This study uses a combination of **activation techniques** and **ion mobility mass spectrometry (IM-MS)** to analyse **gas-phase** ON structures. In this present work, Collision**-Induced Unfolding** (CIU) is used as a "soft" activation method to provide insights into conformational changes upon heating

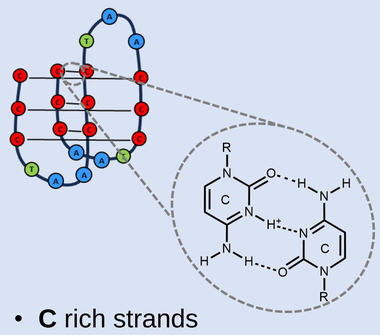
Models

➢ **Three DNA G-quadruplexes** with distinct sequences



- **G** rich strands
- Coordinating cation mediated guanine-guanine interaction

➢ **Intramolecular telomeric i-motif**



- **C** rich strands
- Proton mediated cytosine-cytosine interaction (C-H$^+$-C)

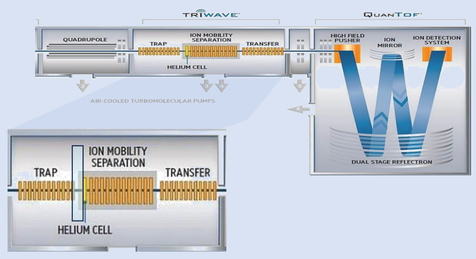
Materials and methods

➢ **Sample preparation**:

- G-quadruplexes→ 10µM of DNA sequence annealed for 7 mins in 100mM NH_4Ac. Addition of 60% ethanol (EtOH) before injection
- I-motif → 25 µM of DNA sequence annealed for 7 mins in 150 mM NH_4Ac pH 5. addition of 15% methanol (MeOH) before injection

➢ **Methods**:

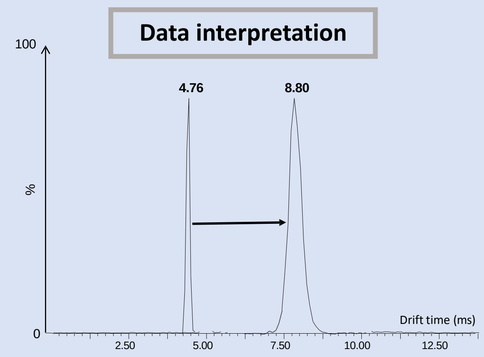
Gas phase activation methods combined **to ion mobility mass spectrometry**
Synapt G2 HDMS (Waters) for the G-quadruplexes and Synapt G2-Si (Waters) for the i-motif
Negative ion mode, cone voltage at 3.0kV, IMS wave conditions: 40V - 1000m/s, N_2 buffer gas 90mL/min



Collision-induced unfolding

Activation of the ion before entering the IM cell ("**slow heating**" activation)

Data interpretation



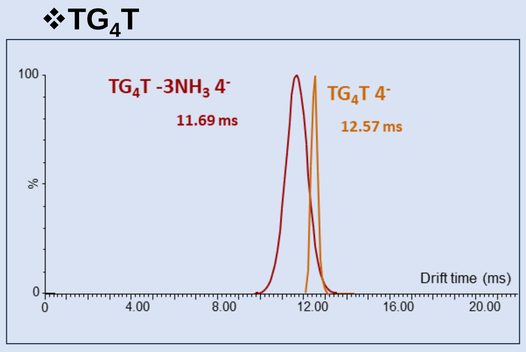
Monitoring of the arrival time distribution (ATD) reflecting the occurrence of potential conformational change upon heating

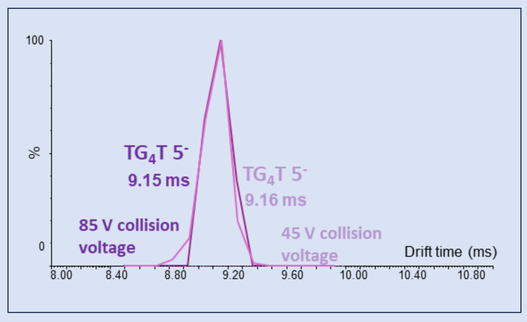
- ➢ shift towards a lower drift time → compaction of the structure
- ➢ Shift towards a higher drift time → unfolding of the structure

Results and discussion

G-quadruplexes

❖**TG_4T**



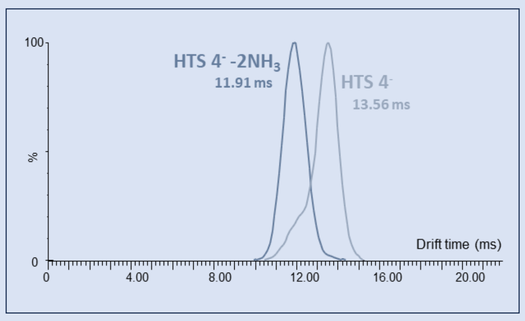


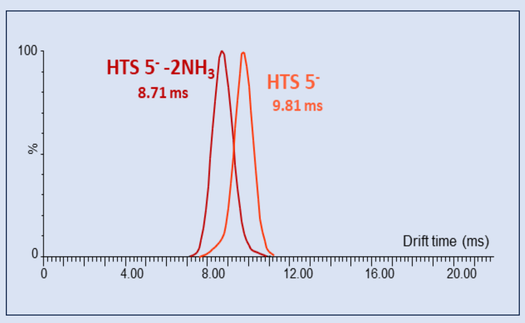
- Lower arrival drift time of GQ **TG_4T (z= -4)** and larger peak width of the arrival time distribution (ATD) after the ejection of 1 coordinating cation

-> partial collapse of the structure and convolution of the ATD due to larger number of co-existing conformers

- GQ **TG_4T (z= -5)** did not lose cations upon heating and maintained the same peak width reflecting a more resilient structure

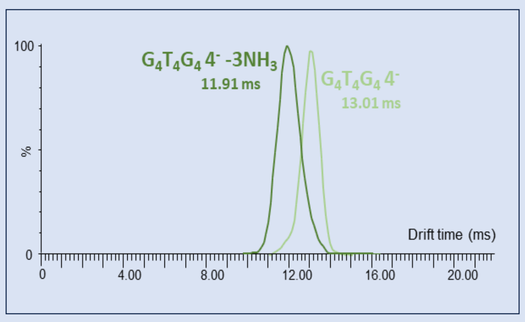
❖**HTS**

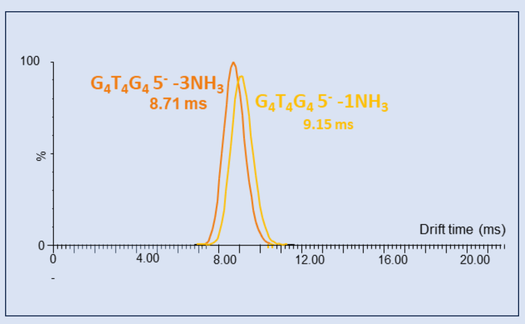




- The **HTS** GQ (both **z= -4 and z= -5**) are more collapsed after the ejection of the coordinating cations

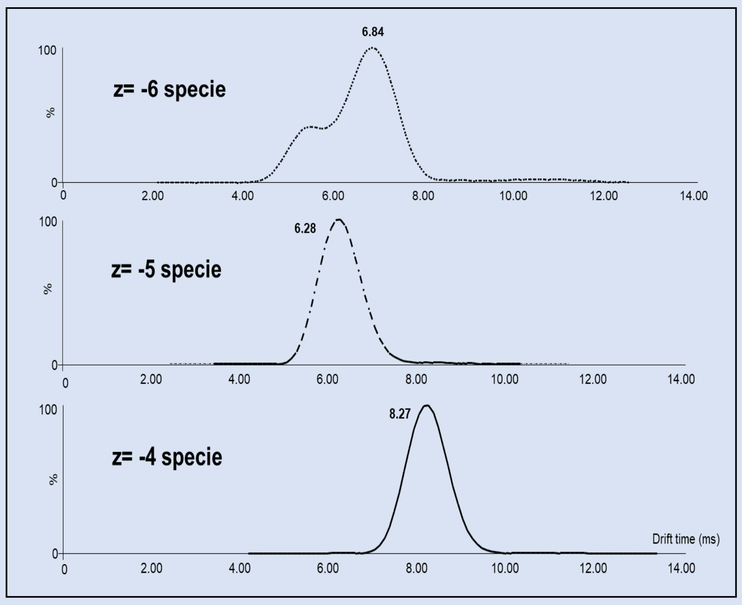
❖**$G_4T_4G_4$**





- Collapse of the structure was also observed for the GQ **$G_4T_4G_4$ (z= -5)** and **$G_4T_4G_4$ (z= -4)** after the ejection of cations upon heating

I-motif



Ion mobility experiments were conducted on the three main charge states

- In a first approximation, we can estimate relative CCS values reported in Table 1 by approximating the drift time value as an ion mobility coefficient and multiplied by the charge according to the Mason-Schamp equation (Equation 1)

→The three charge states exhibited different drift times, with the lowest charge states being more folded than the highest charge being rather in an unfolded state

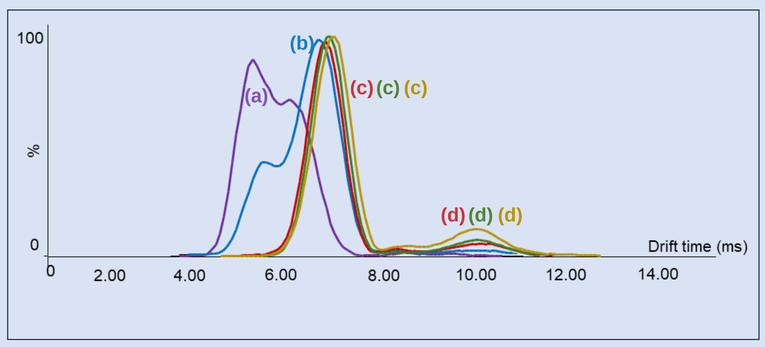
Charge state	Drift time (ms)	Dt*z
-6	6.84 5.14	41 31
-5	6.28	31
-4	8.27	31

Table 1. Conversion of the drift time and charge state to a relative CCS

$$K = \frac{3}{16N}\left(2\frac{\pi}{\mu K_b T}\right)^{1/2} z/\Omega$$

Equation 1. Mason-Schamp equation relating the CCS to the charge

➢ **Collision induced unfolding of the -6 specie**



Bias voltage (V)	Drift time (ms)
25	5.40 (a)
30	6.84 (b)
50	(c) 7.06, 11.02 (d)
70	(c) 7.06, 10.69 (d)
90	(c) 7.06, 10.69 (d)

- At low bias voltage, 2 conformers were detected, one representing a folded structure (at 5.40 ms) and the second one with an extended conformation (at 6.84 ms)
- The increase of the bias voltage produced extended conformers upon activation

Conclusion and perspectives

❖ The results showed that the two models underwent different conformational changes after heating, most probably due to the different HOS, transitioning from an extended state to a more compact state for the GQ, due to the loss of cations and rather an unfolding of the i-motif structure due to the loss of the C-H+-C interaction

- Generation of CIU heatmaps for the z=-6 specie of the i-motif and conduction of CIU experiments and generation of CIU heatmaps for the z=-5 specie. How does the charge state affect the conformational evolution of the system upon heating?
- Conduction of CID experiments and investigation of fragmentation pathways for the i-motif and GQs.

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