Higher order structures of G-quadruplexes and DNA duplexes investigated by ion mobility mass spectrometry, breakdown curve experiments and collision induced unfolding

Zeina Hajjar[a], Cédric Delvaux[a], Maxime Benonit[a], Christopher Kune[a], Thomas De Vijlder[b], Laure-Elie Carloni[b], Gauthier Eppe[a], Edwin De Pauw[a] and Johann Far[a]

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

[b] Pharmaceutical Development & Manufacturing Sciences (PDMS), Janssen Research & Development, Beerse, Belgium

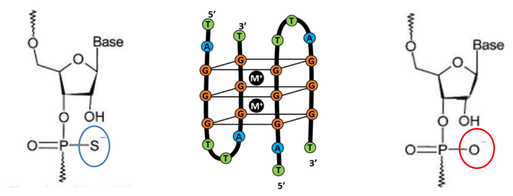
Z.Hajjar@uliege.be

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. **Collision-Induced Unfolding (CIU)** and **Collision-Induced Dissociation (CID)** are respectively used as "soft" and "hard" activation methods to provide insights into conformational changes and fragmentation pathways through **CIU heatmaps and breakdown curve plots.**

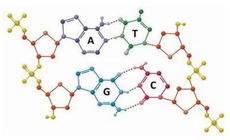
Higher order structure models

- NH_4^+ and K^+ **DNA G-quadruplexes** with phosphodiester and phosphorothioate backbones



- Five **DNA duplexes** with increasing GC base pair percentage

GC%	Constitutive strands
0%	AAATTATAATATTAAA TTTAATATTATAATTT
25%	GGATTATAATATTAGG CCTAATATTATAATCC
50%	GGGCTATAATATCGGG CCCGATATTATAGCCC
75%	GGGCCGTAATGCCGGG CCCGGCATTACGGCCC
100%	GGGCCGCGGCGCCGGG CCCGGCGCCGCGGCCC



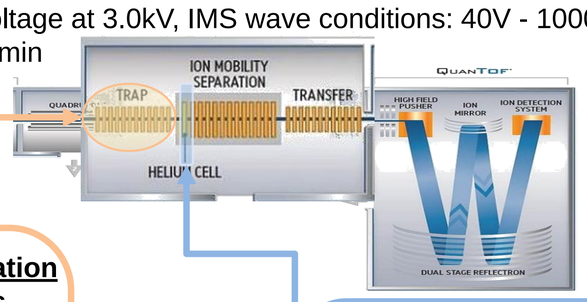
Materials and methods

- **Sample preparation**:

G-quadruplexes→ 10µM $(TAGGGT)_2$ in 100mM NH_4Ac, 60% Ethanol (EtOH)
DNA duplexes→ DNA single strands were annealed in NH_4Ac/MeOH (80:20) to form duplexes

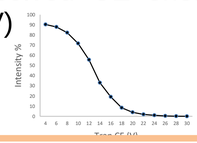
- **Methods**:

Gas phase activation methods combined **to ion mobility mass spectrometry**
Synapt G2 HDMS (Waters)
Negative ion mode, cone voltage at 3.0kV, IMS wave conditions: 40V - 1000m/s N_2 buffer gas 90 mL/min, He: 180 ml/min



Collision-induced dissociation and breakdown curves

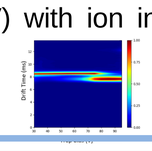
Dissociation of the ion via a collision with argon ("**hard**" activation)→ survival yields at resolved CE energy trap CE (V)



Different energy regimes so V_{50} values cannot be compared

Collision-induced unfolding and CIU heatmaps

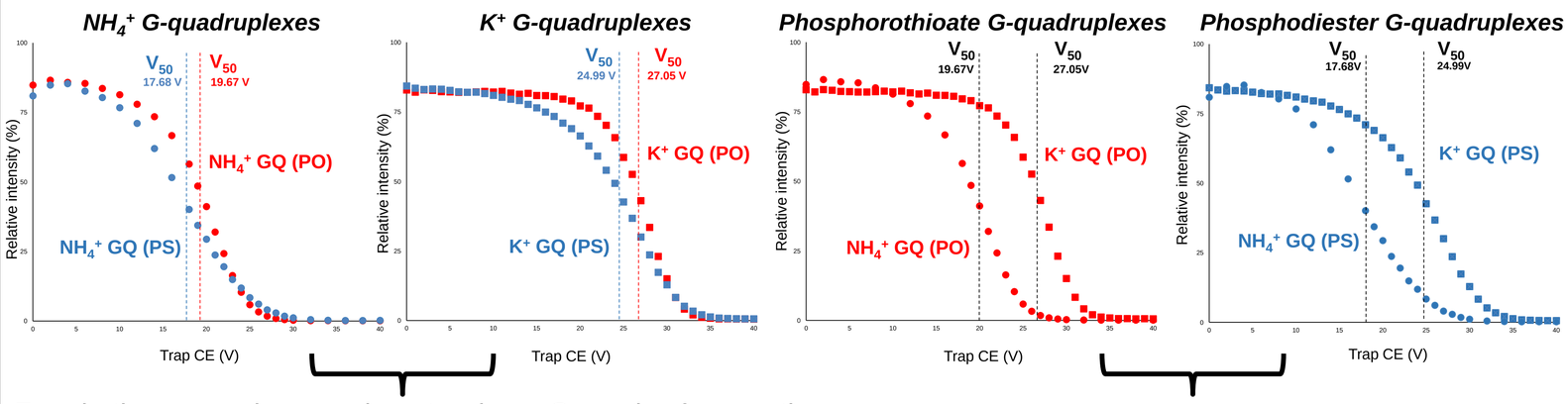
Acceleration voltage (He/N_2) for injection inside IM cell (N_2) ("**slow heating**" activation)→ drift time evolution at different Trap bias (V) with ion intensity color coded



Results and discussion

G-quadruplexes

❖ CID experiments

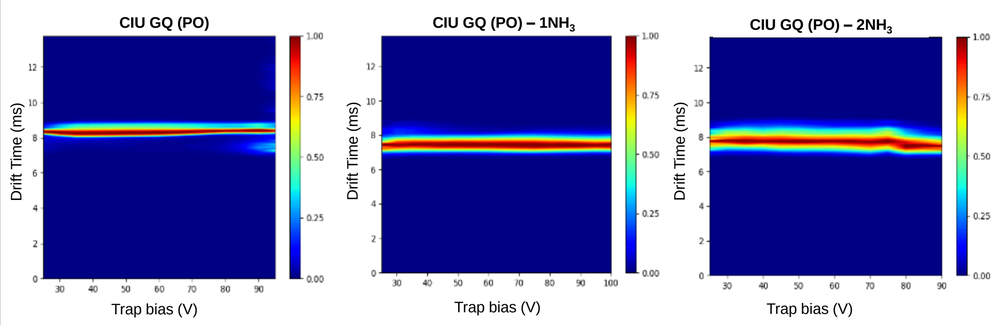


For both ammonium and potassium G-quadruplexes, the conversion of the classical phosphodiester backbone to a phosphorothioate backbone exhibits lower V_{50} values suggesting a lower stability in the gas phase

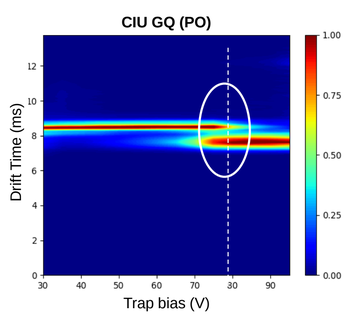
Ammonium species exhibit lower V_{50} values than the K^+ GQ. Main fragmentation channel of the NH_4^+ GQ is the loss of a neutral ammonia molecule

❖ CIU experiments

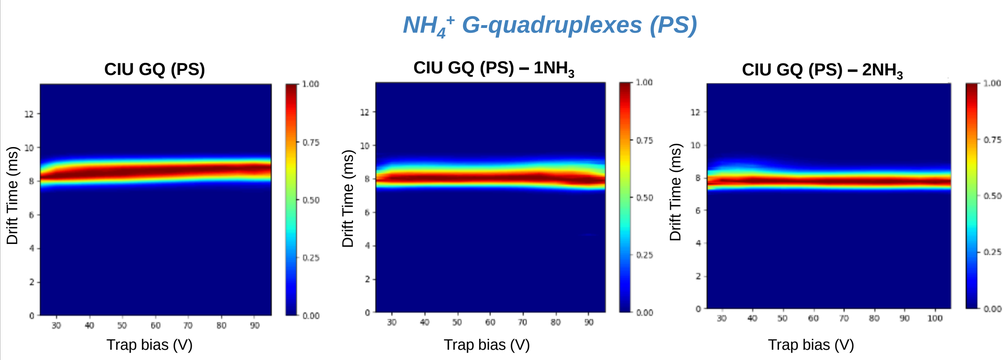
NH_4^+ G-quadruplexes (PO)



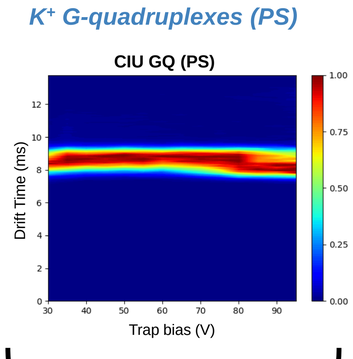
K^+ G-quadruplexes (PO)



NH_4^+ G-quadruplexes (PS)



K^+ G-quadruplexes (PS)

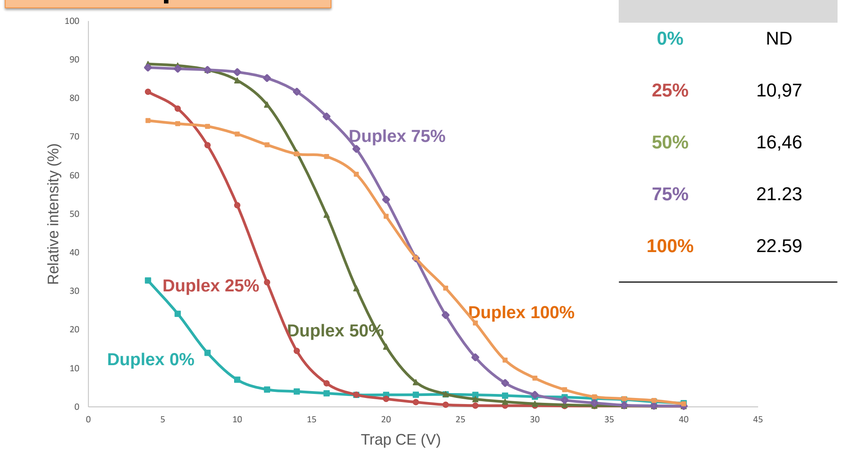


Ammonium-based G-quadruplexes exhibit a progressive shift towards lower drift times upon the loss of NH_3, indicative of a structure compaction

Potassium-based G-quadruplexes exhibit a remarkable shift towards lower drift times, especially the phosphodiester, indicative of a structure compaction without cation ejection

DNA duplexes

❖ CID experiments

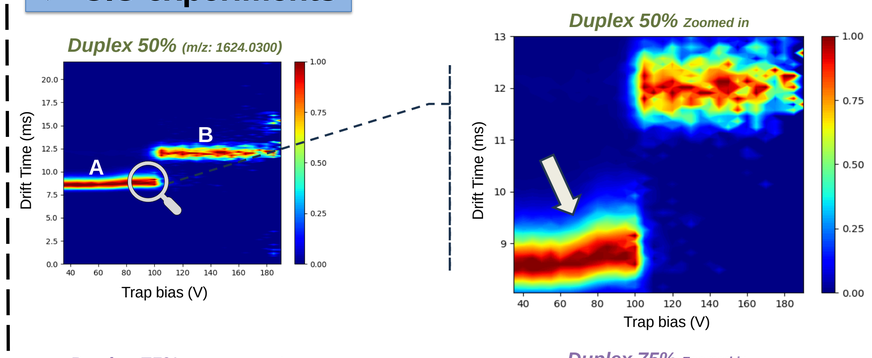


Duplex 6^-	V_{50} value
0%	ND
25%	10,97
50%	16,46
75%	21.23
100%	22.59

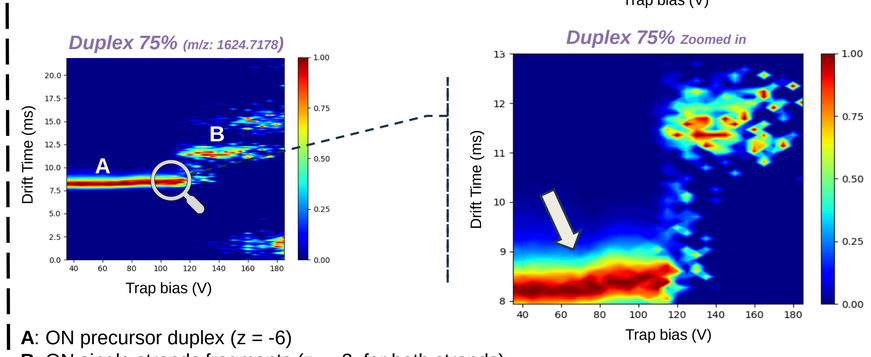
V_{50} values increase with the increase of GC % content in the duplex sequences → effect of the GC base pairing (number of hydrogen bonds) on the duplex resilience to dissociation.
Explored fragmentation pathways sequence dependent?

❖ CIU experiments

Duplex 50% (m/z: 1624.0300) — *Duplex 50% Zoomed in*



Duplex 75% (m/z: 1624.7178) — *Duplex 75% Zoomed in*



A: ON precursor duplex (z = -6)
B: ON single strands fragments (z = -3, for both strands)

The CIU heatmaps suggest the occurrence of some possible conformational change within the duplex before its fragmentation into its respective single strands. Data processing and further experiments are being conducted to confirm the (un)folding and fragmentation pathways when submitted to a slow heating activation

Conclusion, perspectives and acknowledgments

❖ **G-quadruplexes**

CID⇒ Substitution from PO to PS backbone in both NH_4^+ and K^+ GQ lowered their V_{50} values→ reduced gas phase stability. The decrease in V_{50} values in NH_4^+ species is due to the loss of a neutral ammonia molecule.

CIU⇒ NH_4^+ GQ exhibited a progressive drift time shift upon the loss of NH_3 whereas K^+ species exhibited a remarkable shift towards lower drift times, especially in the phosphodiester species →structural compaction in both cases (without cation ejection for the K^+ species).

❖ **DNA duplexes**

CID⇒ Increasing the GC % within the duplex sequence leads to a gradual increase in V_{50} values → impact GC base pairs on duplex stability.

CIU⇒ possible conformational change of the duplex before its fragmentation into single strands.

Next steps?

- Further analysis of the duplex's behavior upon heating
- Stability and conformational change study in solution and comparison with the gas phase results… correlation?

fnrs LA LIBERTÉ DE CHERCHER
janssen PHARMACEUTICAL COMPANIES OF Johnson&Johnson