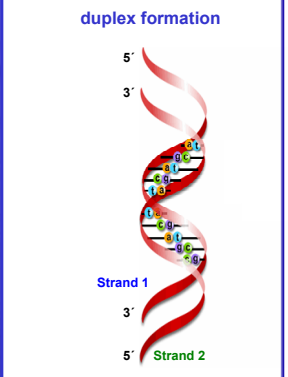
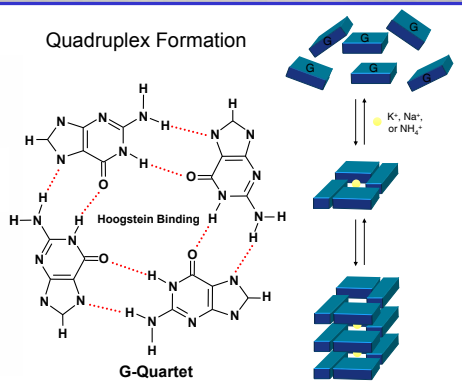
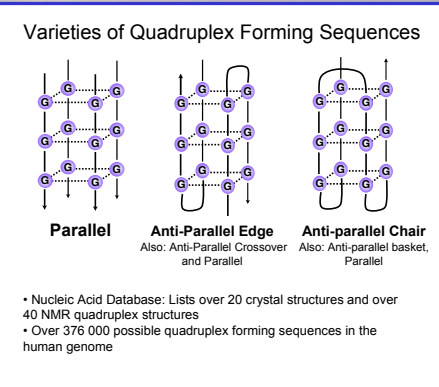
INTRODUCTION

Recently, interest in G-quadruplex structures has increased greatly due to the discovery of many G-rich biologically significant regions present in the human genome. These regions include the immunoglobulin switch region, gene promoter regions, sequences associated with various human diseases and in telomeres at the end of chromosomes.[1-5] It has been postulated that G-rich segments of the HIV-1 Central Flap Sequence can form quadruplex structures and that they can play a role in regulating the reverse transcription process necessary for host cell infection.[5] Our goal is to determine if this particular HIV-1 DNA sequence forms quadruplex structures and to try to target the quadruplex with stabilizing ligands. An electrospray ionization source coupled to an ion mobility mass spectrometer was used to acquire mass spectra and measure collisional cross-sections of ODNt+, our quadruplex model d[T(G)$_6$TACA(G)$_4$A], which was designed to mimic the structural features of the biologically relevant central flap sequence. The experiments on ODNt+ when compared to the previously studied human telomeric sequence, T2, indicate that two stranded quadruplex structures are stable and are competitive even in the presence of the complimentary strand.

DNA BACKGROUND

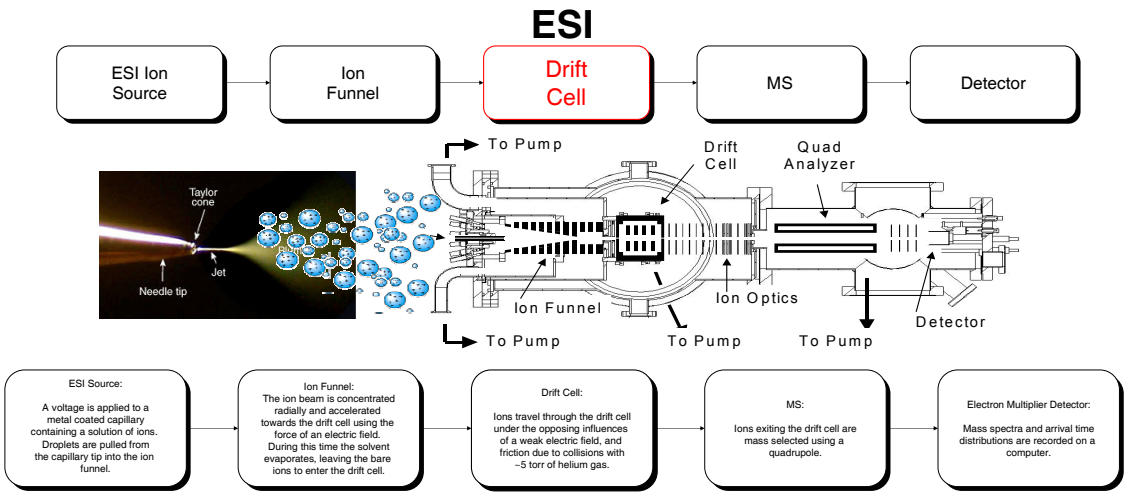




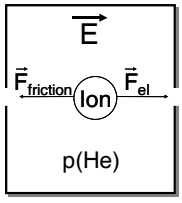


- Nucleic Acid Database: Lists over 20 crystal structures and over 40 NMR quadruplex structures
- Over 376 000 possible quadruplex forming sequences in the human genome

METHODS



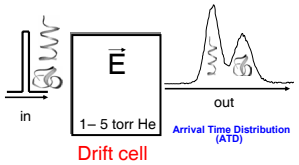
$\vec{v}$ = const.
$\vec{v}$ = K E
K = ion mobility



Concepts of Ion Mobility

Get shape information from ion mobility

Time is measured starting from when the ions are pulsed into the drift cell, until they reach a detector. Inside the drift cell they encounter ~5 torr of helium gas. Larger structures will experience more collisions with the He, increasing the frictional drag, and lengthening the amount of time they spend in the cell, as compared to a more compact structure. This enables us to temporally separate structures of different collisional cross-sections.



$K = f(T, p, q, \mu, \sigma)$

T = temperature
p = pressure
q = ion charge
μ = reduced mass
σ = collision cross section

ion shape

DATA ANALYSIS

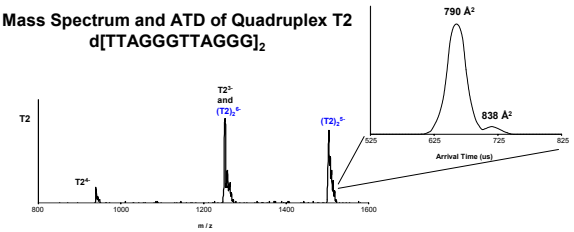
Experimental Method → **ARRIVAL TIME DISTRIBUTIONS (ATDs)** → **MOBILITIES** → **COLLISION CROSS-SECTIONS**

Compare

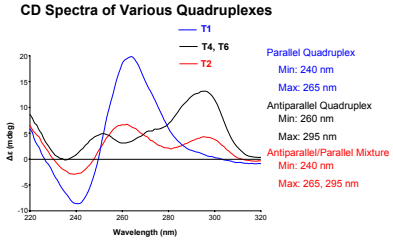
Theoretical Method → **MOLECULAR MECHANICS/DYNAMICS** → **STRUCTURES** → **COLLISION CROSS-SECTIONS**

Two Stranded Quadruplex Formation in Human Telomeric Sequence T2

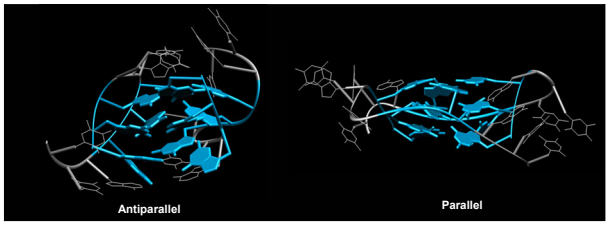
Mass Spectrum and ATD of Quadruplex T2 d[TTAGGGTTAGGG]$_2$



CD Spectra of Various Quadruplexes



- The mass spectrum of T2 shows stoichiometries consistent with two stranded quadruplex structures.
- Two stable structures are present in the ATD of the $(T2)_2^{-5}$ complex.
- The CD spectrum of the T2 solution shows features that are consistent with the presence of both parallel and anti-parallel structures.



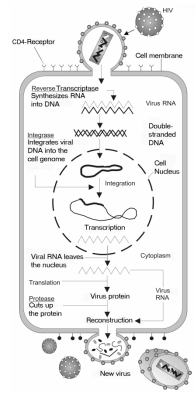
T2 Form	Calculated Cross Section (Å²)
Globular	745
Anti-parallel Edge	785
Anti-parallel Crossover	788
Parallel Propeller	845

σ_{exp} = 790 Å²

σ_{exp} = 838 Å²

- The calculated cross sections of the two forms of anti-parallel quadruplex are too close to be able to distinguish which form is present in experiment.
- The modeling shows that the larger structure in the $(T2)_2^{-5}$ ATD corresponds to the parallel propeller quadruplex form whereas the smaller structure is a form of anti-parallel quadruplex.
- The calculated cross section of the globular form of the $(T2)_2^{-5}$ complex indicates that it is not present in our experiment

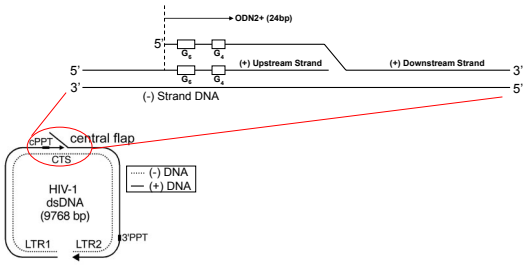
HIV-1 Introduction



Review of HIV Infection of Host Cell

1. Glycoprotein on surface of HIV-1 viron interacts with receptor on the surface of the host cell.
2. Induces injection of viral genome and other particles (proteins) into the host cell.
3. Reverse Transcriptase makes copy of the RNA(+) strand called DNA(-) strand.
4. Reverse Transcriptase then makes the DNA(+) strand to create the viral DNA intermediate vDNA.
5. vDNA is transported into the nucleus of the host cell and incorporated into the host genome by Integrase.
6. It waits and is regulated by other proteins until the signal comes to start making more RNA viral genomes to be packaged and assembled.

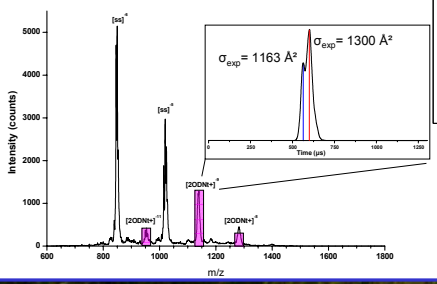
Possible Quadruplex Forming Sequences



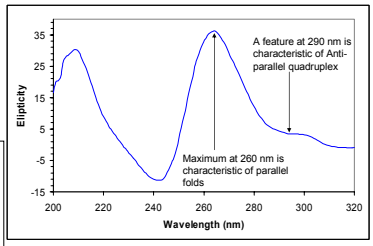
ODN2+: d[TT$(G)_6$TACAGTGCA$(G)_4$AAA] → Biological

ODNt+: d[T$(G)_6$TACA$(G)_4$A] → Model

ODNt+: d[TGGGGGGTACAGGGGA]
Sample Prep: Short Annealing Time (3-20min)

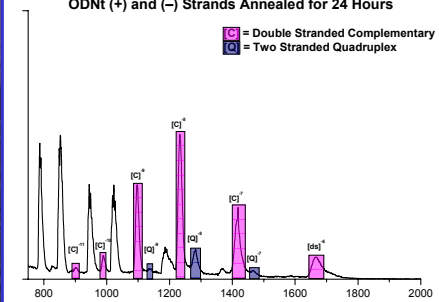


CD Spectra of ODNt+ with a short annealing time



- The mass spectrum shows species are retained in the gas phase that are indicative of quadruplex formation.
- Two stable structures are visible in the ATD which is in agreement with the ATD of the similar quadruplex T2.
- The presence of two stable structures is also indicated in the CD spectrum with two features; one at 260 nm which is characteristic of parallel folds and one at 290 nm which is characteristic of Anti-parallel folds

ODNt (+) and (–) Strands Annealed for 24 Hours



- Significant quantities of both complementary double stranded species and two stranded quadruplex species are present in solution
- It is possible that an equilibrium between the two stranded complementary and two stranded quadruplex species exists
- We can also try to stabilize the quadruplex with specific ligands and try to favor the quadruplex species over complementary species

CONCLUSIONS

1. **It has been shown that the two repeat Human Telomeric sequence, which is analogous to the HIV-1 central flap sequence, forms stable parallel and anti-parallel quadruplex structures in solution that are retained in the solvent free environment.**

2. **Stoichiometries in the mass spectrum, structures in the ATD, and features in the CD spectrum, indicate that the model sequence designed to mimic the biological HIV-1 central flap sequence forms two stable, two stranded quadruplex species. This is in agreement with the T2 quadruplex.**

3. **When annealed in the presence of the complementary (-) DNA strand there are two types of complexes present in the mass spectrum. The two stranded quadruplex species is competitive with the complimentary double stranded species under our annealing conditions.**

4. **Future studies with different model sequences should improve the ease of modeling permitting assignment of ATD structures. In addition we can now start to look for ligands that bind specifically to quadruplex structures with the hope of stabilizing the quadruplex.**

References

1. Richard R. Sinden. **DNA Structure and Function.** Academic Press, San Diego, CA. 1994
2. Baker, E.S., Bernstein, S.L., Gabelica, V., De Pauw, E., Bowers. M.T. **G-quadruplexes in telomeric repeats are conserved in a solvent-free environment.** *Int. J. Mass Spec.* 2006, 253, 225-237.
3. Huppert, J.L., Balasubramanian, S. **Prevalence of quadruplexes in the human genome.** *Nucleic Acids Research.* 2005, Vol. 33, No. 9, 2908-2916.
4. Burge, S., Parkinson, G.N., Hazel, P., Todd, A.K., Neidle, S. **Quadruplex DNA: sequence, topology and structure.** *Nucleic Acids Research.* 2006, Vol.43, No.19, 5402-5415.
5. Lyonnaise, S., Hounsou, C., Teulade-Fichou, MP., Jeusset, J., Le Cam, E., Mirambeau, G. **G-quartets assembly within a G-rich DNA flap. A possible event at the center of the HIV-1 genome.** Nucleic Acids Research. 2002, Vol. 30, No. 23, 5276-5283.
6. http://bowers.chem.ucsb.edu