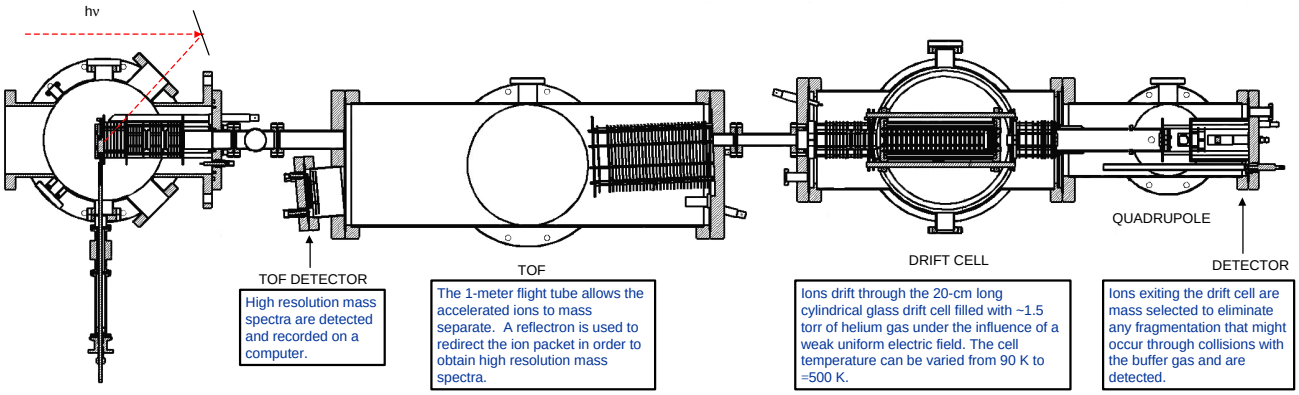
## ABSTRACT

The aggregation and conformations of sodiated dinucleotides were examined by mass spectrometry, ion mobility and molecular modeling methods. Single-strand, duplex and triplex ions of the form $[M + nNa - (n-1)H]^+$ with $n$= 1-10 were observed in the MALDI-TOF mass spectra. Deprotonation sites were determined to be on the phosphates and the thymine and guanine bases. Collision cross-sections of each sodiated complex were measured using ion mobility methods and compared to calculated cross-sections of theoretical structures generated by molecular dynamics (AMBER). Three distinct single-strand conformers are observed: one with the nucleobases stacked, one with the bases perpendicular to each other and one with the bases coplanar. As more $Na^+$ ions attach to the single-strands, the dinucleotides tend to favor the stacked form. The duplexes and triplexes are held together via $Na^+$ bridging between the deprotonated phosphates, eventually forming a $Na^+$-O ring. Additional $Na^+$ ions that cannot fit in the ring bind to various places on the bases. Dinucleotides with guanine bases do not form triplexes, presumably because the guanines stack in such a way that the phosphates cannot be bridged together with $Na^+$ ions.

## METHODS

### MALDI-TOF




**SOURCE**: A molecule of interest is dried on a stainless steel rod. The rod is inserted into the source chamber and a nitrogen laser is used to desorb and ionize the molecule.

**TOF DETECTOR**: High resolution mass spectra are detected and recorded on a computer.

**TOF**: The 1-meter flight tube allows the accelerated ions to mass separate. A reflectron is used to redirect the ion packet in order to obtain high resolution mass spectra.

**DRIFT CELL**: Ions drift through the 20-cm long cylindrical glass drift cell filled with ~1.5 torr of helium gas under the influence of a weak uniform electric field. The cell temperature can be varied from 90 K to ≈500 K.

**DETECTOR**: Ions exiting the drift cell are mass selected to eliminate any fragmentation that might occur through collisions with the buffer gas and are detected.

### EQUATIONS

$$K_o = \left( l^2 \cdot \frac{273}{760T} \cdot \frac{p}{V} \cdot \frac{1}{t_A - t_o} \right)$$

The reduced mobility, $K_o$, is obtained from ATDs using the equation above where $l$ is the length of the cell, $T$ is the temperature in Kelvin, $p$ is the pressure of the He gas (in torr), $V$ is the strength of the electric field, $t_A$ is the ions' arrival time taken from the center of the ATD peak, and $t_o$ is the amount of time the ion spends outside the drift cell before reaching the detector.

$$s = \frac{3e}{16N_o}\left(\frac{2p}{mk_bT}\right)^{1/2}\frac{1}{K_o}$$

The ion's collision cross-section, $s$, is calculated using the equation above where $e$ is the charge of the ion, $N_o$ is the number density of He at STP, $k_b$ is Boltzmann's constant, and $m$ is the ion-He reduced mass.

### DATA ANALYSIS

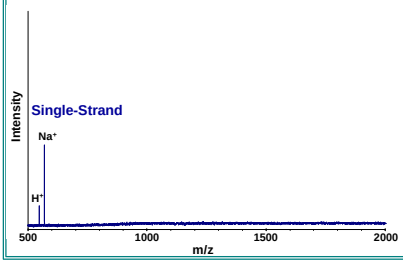
Experimental Method: **ARRIVAL TIME DISTRIBUTIONS (ATDs)** → **MOBILITIES** → **COLLISION CROSS-SECTIONS**

Compare

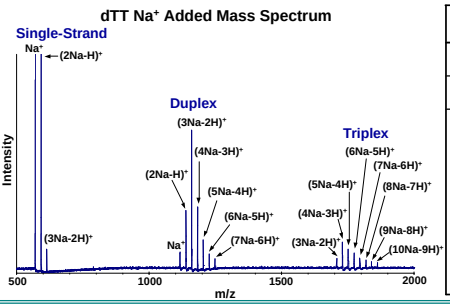
Theoretical Method: **MOLECULAR MECHANICS/DYNAMICS** → **STRUCTURES** → **COLLISION CROSS-SECTIONS**

# $Na^+$Dinucleotide Mass Spectra Analysis

### dTT No $Na^+$ Added Mass Spectrum




### dTT $Na^+$ Added Mass Spectrum




| Maximum Number of $Na^+$ | | | | |
|---|---|---|---|---|
| Single-Strand | | Duplex | | |
| 2 | 3 | 3 | 5 | 7 |
| dAA<br>dCA<br><br>dAC<br>dCC | dGA<br>dTA<br>dGC<br>dTC<br><br>dAG<br>dCG<br>dGG<br>dTG<br><br>dAT<br>dCT<br>dGT<br>dTT | dAA<br>dCA<br><br>dAC<br>dCC | dGA<br>dTA<br>dGC<br>dTC<br><br>dAG<br>dCG<br>dAT<br>dCT | dGG<br>dTG<br>dGT<br>dTT |

This table illustrates the maximum number of $Na^+$ that will bind to each dinucleotide single-strand or duplex. For each G or T in the dinucleotide sequence, an extra sodium will be added due to the acidic hydrogens on G and T.


Adenine

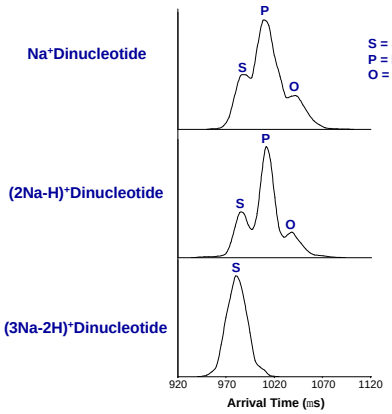

Cytosine


Guanine


Thymine

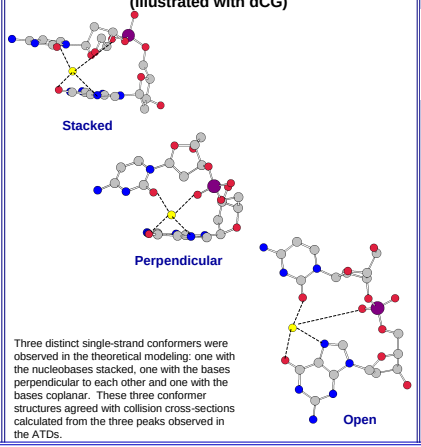
## Sodiated Single-Strands

### 80K Sodiated Single-Strand ATD Trend



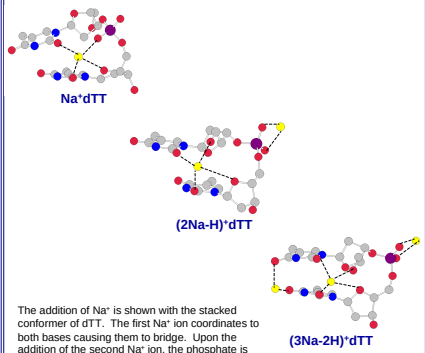

S = Stacked
P = Perpendicular
O = Open

### Na+ Single Strand Theoretical Families (illustrated with dCG)




Three distinct single-strand conformers were observed in the theoretical modeling: one with the nucleobases stacked, one with the bases perpendicular to each other and one with the bases coplanar. These three conformer structures agreed with collision cross-sections calculated from the three peaks observed in the ATDs.

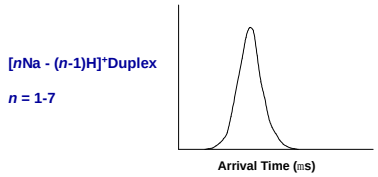
### Effect of Sodiation on Stacked Conformation




The addition of $Na^+$ is shown with the stacked conformer of dTT. The first $Na^+$ ion coordinates to both bases causing them to bridge. Upon the addition of the second $Na^+$ ion, the phosphate is deprotonated, so the second $Na^+$ binds to the phosphate group. When three $Na^+$ ions are added, a base must be deprotonated. This allows two $Na^+$ ions to coordinate to the bases and one to bind to the phosphate group. The trend is similar for the perpendicular and open conformers.

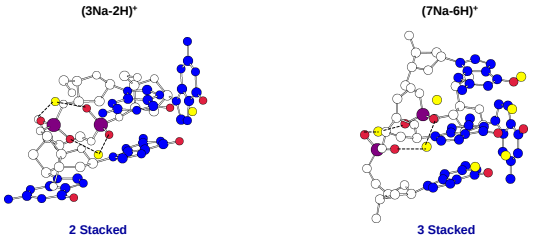
## Sodiated Duplexes

### 80K Sodiated Duplex ATD Trend




One ATD peak was observed in all duplex systems.

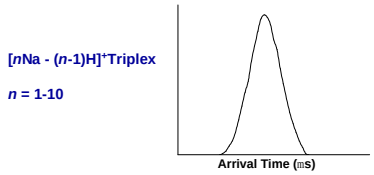
### Sodiated dGG Duplex Theoretical Structures




Duplexes are held together by $Na^+$ ions bridging the deprotonated phosphates. As additional $Na^+$ ions are added, they begin to bind to various places on the bases, since they can no longer fit in the ring. Only phosphates and atoms which participate in $Na^+$ binding are illustrated in the theoretical structures.

## Sodiated Triplexes

### 80K Sodiated Triplex ATD Trend

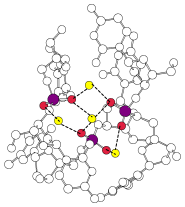



Only one ATD peak was illustrated for all triplex systems.

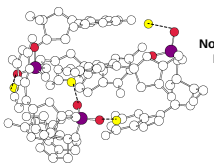
| $Na^+$Triplex Formation | |
|---|---|
| Triplex | No Triplex |
| dAA<br>dCA<br>dTA<br>dAC<br>dCC<br>dTC<br>dAG<br>dAT<br>dCT<br>dTT | dGA<br>dGC<br>dCG<br>dGG<br>dTG<br>dGT |

This table illustrates that triplex formation is inhibited by dinucleotide sequences containing G. G induces stacking, which does not allow $PO_4$ groups to cluster as shown in the theoretical structure.

### Sodiated Triplex Theoretical Structures




$s_{EXPT}$ = 297 Å²
$s_{Theory}$ = 297 Å²




Not Observed in Experiment

Triplexes are held together by $Na^+$ ions bridging deprotonated phosphates. Dinucleotides containing guanine do not form triplexes because guanines stack in such a way that the phosphates cannot be bridged together. The theoretical structures are shown with all atoms whited out except sodium, phosphate and phosphate oxygens to clearly illustrate triplex clustering.

## CONCLUSIONS

1. Dinucleotides form duplexes and triplexes in the gas phase upon addition of $Na^+$
2. In all cases, the maximum number of $Na^+$ ions attached equals the number of acidic hydrogens plus one. T and G are the only bases that deprotonate in the presence of $Na^+$ ions.
3. 3 structural families are observed for the single-strand systems: bases stacked, bases perpendicular or bases planar. The addition of $Na^+$ ions cause the single-strand to favor the formation of the stacked conformer.
4. The duplexes and triplexes are held together by $Na^+$-O bridging between the phosphate groups.
5. G hinders triplex formation, presumably due to its strong inclination for base stacking.