

# **Ammonium ion binding to DNA G-quadruplexes: do electrospray mass spectra faithfully reflect the solution-phase species?**

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## S1. Sample preparation

All DNA strands were purchased from Eurogentec (Seraing, Belgium) with RP-cartridge/Oligold purity. Lyophilized oligonucleotides were resuspended in nuclease-free H<sub>2</sub>O (Ambion). The strand concentrations were determined by absorbance spectroscopy, using the molar extinction coefficients listed in Table 1, then diluted to obtain final stock solutions of 100  $\mu$ M single strand in 150 mM aqueous NH<sub>4</sub>OAc. Absorbance measurements were done using a Uvikon XS (Secomam, Alès, France). These solutions were heated to 80°C and cooled overnight to room temperature. Solutions were then stored at 4°C until used for ESI-MS (at least 12 hours). Before measurement, the stock solutions were brought to room temperature (22°C) and diluted to 10  $\mu$ M strand concentrations with 150 mM aqueous NH<sub>4</sub>OAc. At these concentrations, the melting temperatures of the G-quadruplexes, determined from absorbance variations at 295 nm, range from 35°C (dG<sub>3</sub>T<sub>4</sub>G<sub>4</sub> and TBA) to 70°C (dG<sub>4</sub>(T<sub>4</sub>G<sub>4</sub>)<sub>3</sub>). Because organic co-solvents could shift the solution-phase equilibria and cause structural changes [1-4], 100% aqueous solutions were used for mass spectrometry.

**Table S1:** Molar extinction coefficients of sequences used in the present study

sequence	$\epsilon_{260}$ (M <sup>-1</sup> cm <sup>-1</sup> )
dG <sub>4</sub> T <sub>4</sub> G <sub>4</sub>	115 200
dG <sub>4</sub> T <sub>3</sub> G <sub>4</sub>	107 100
dG <sub>3</sub> T <sub>4</sub> G <sub>4</sub>	105 100
dG <sub>4</sub> (T <sub>4</sub> G <sub>4</sub> ) <sub>3</sub>	262 000
dG <sub>2</sub> T <sub>2</sub> G <sub>2</sub> TGTG <sub>2</sub> T <sub>2</sub> G <sub>2</sub>	143 300

## S2. Optimized ESI-MS tuning parameters

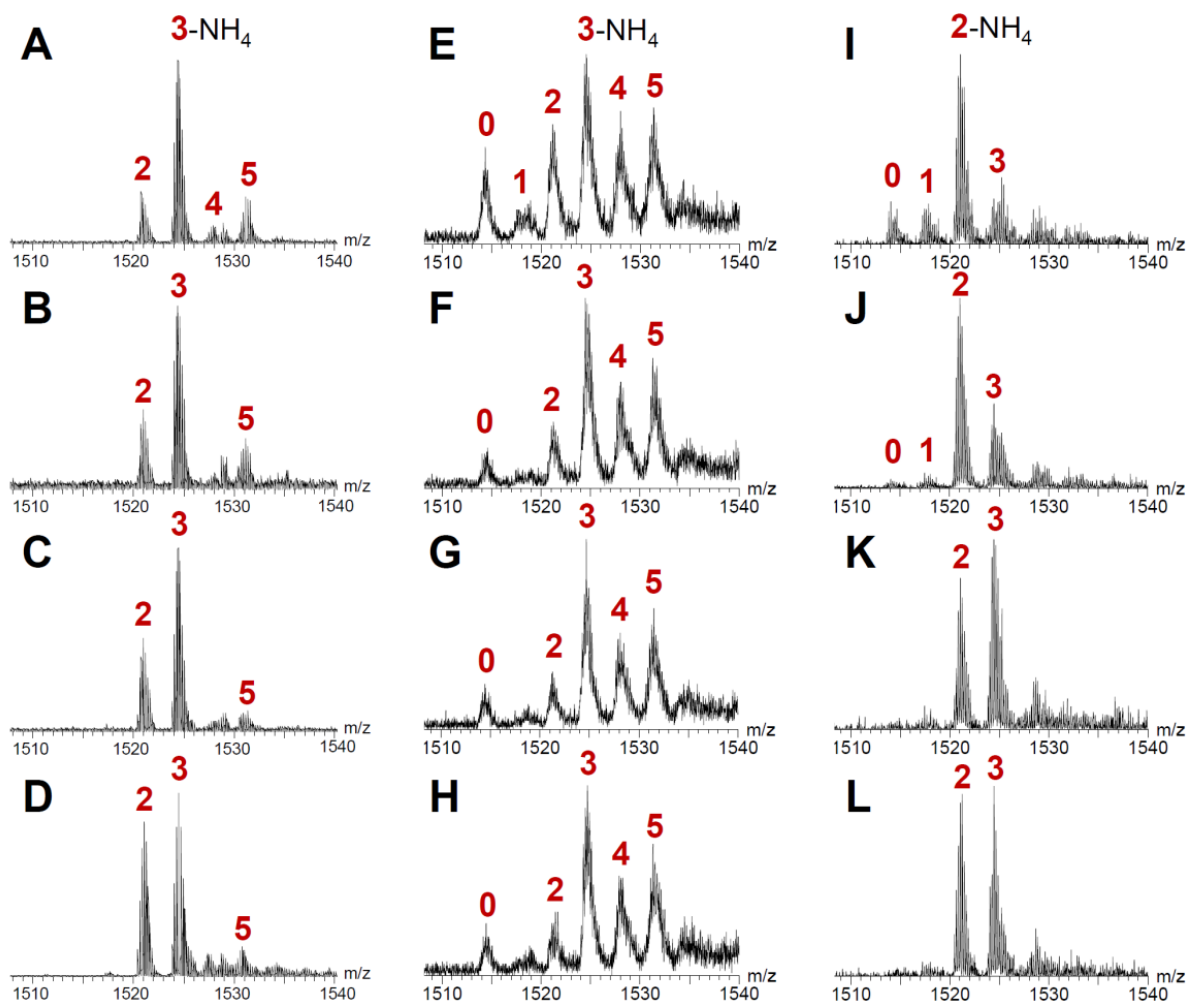
**On the Solarix 9.4T ESI-Q-FTICRMS** (Bruker Daltonics, Bremen, Germany), we used a drying gas temperature of 20 °C (critical parameter), a capillary exit voltage of -180 V, a skimmer 1 voltage of -5 V (spectra are identical from -5 V to -30 V, and change beyond this value), a skimmer 2 voltage of -4 V, and a collision voltage of 0 V (critical parameter). The ammonium ion distribution of the bimolecular quadruplex  $[(dG_4T_4G_4)_2]^{5-}$  in these soft conditions is shown in Figure S2A. The most critical parameters were the skimmer 1 voltage (ammonium ion loss increases starting at -40 V, see Fig. S2B), the collision energy (Fig. S2C), and the drying gas temperature (compare Fig. S2D at 60 °C with Fig. S2C at 20 °C). Note that temperatures traditionally used for most analytes are in the range of 200 °C, according to the manufacturer's recommendations.

**On the Q-TOF Ultima Global ESI-Q-TOFMS** (Waters, Manchester, UK), we used a source pressure of 3.43 mbar, a cone voltage between 35 V and 100 V, an RF Lens 1 voltage between 0 V and 60 V (critical parameter beyond that value), a collision energy of 2 V (critical parameter), and source/desolvation temperatures of 20 °C (critical parameter). On the Q-TOF Ultima Global, the source and desolvation temperatures significantly influence the detected ammonium ion distribution (compare Fig. S2E and Fig. S2F). In Fig. S2F, the 3-NH<sub>4</sub>/2-NH<sub>4</sub> ratio is similar to that obtained on the ESI-FTICRMS (Fig. S2A), but simultaneously, a peak with zero ammonium ion adducts is also observed. Further decreasing the cone voltage (Fig. S2G) and the RF Lens 1 voltage (Fig. S2H), did not suppress this peak.

**On the Synapt G1 HDMS ESI-Q-IM-TOFMS** (Waters, Manchester, UK), we used a source pressure of 3.15 mbar, a sampling cone voltage of 20 V (critical parameter), an extraction cone voltage of 4 V, source and desolvation temperatures of 40 °C, trap and transfer collision energies of 2 V (critical parameter), a trap DC bias voltage of 8 V (critical parameter), an IMS pressure (nitrogen) of 0.532 mbar, and waves of 8V at 300 m/s.

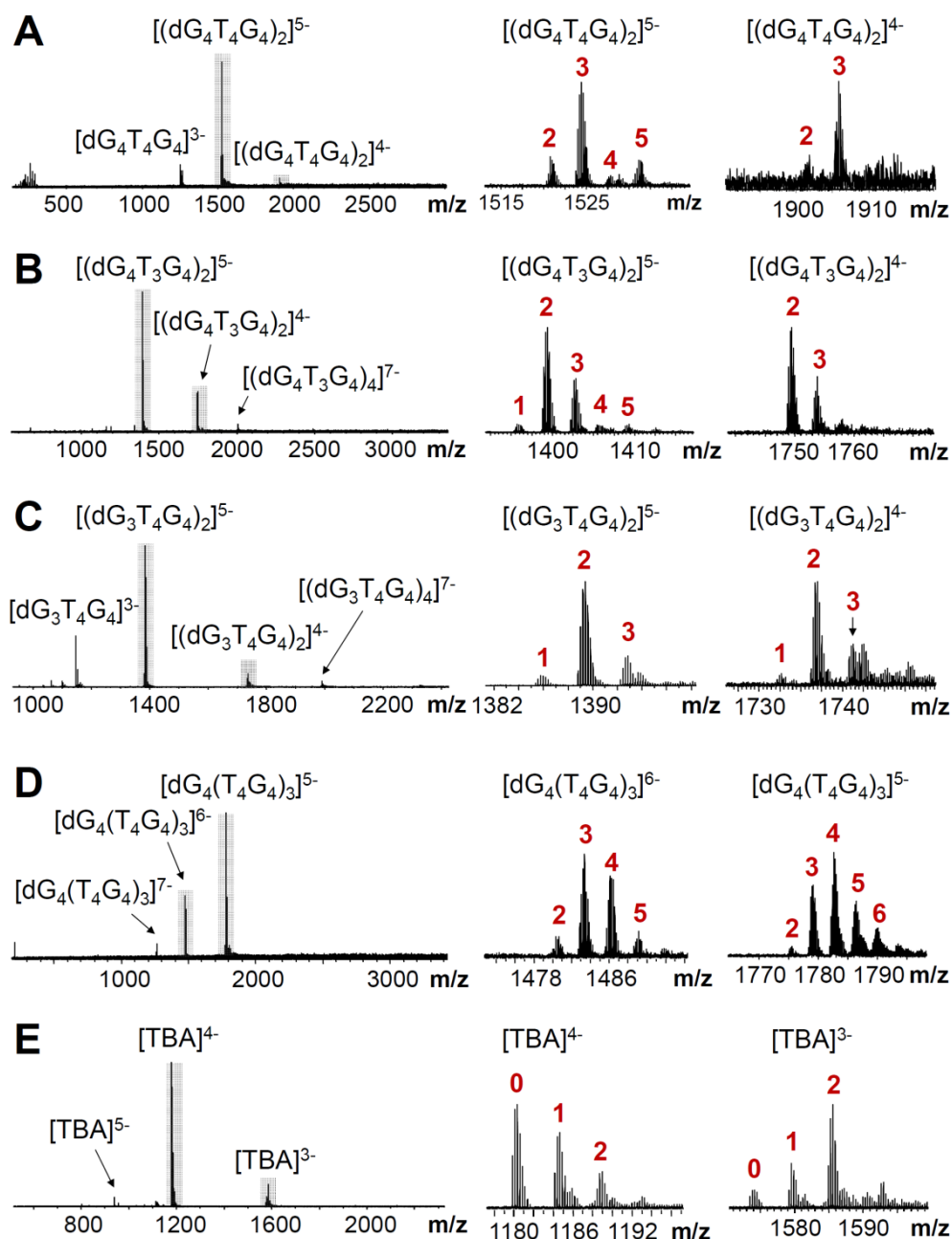
**On the Synapt G2 HDMS ESI-Q-IM-TOFMS** (Waters, Manchester, UK), we used a source pressure of 2.82 mbar, a sampling cone voltage of 20 V (critical parameter), an extraction cone voltage of 2 V, source and desolvation temperatures of 30 °C and 40 °C respectively, trap and transfer collision energies of 4 V and 0 V respectively, a trap DC bias voltage of 35 V (critical parameter), an IMS pressure (nitrogen) of 1.820 mbar (Helium cell gas flow of 180 mL/h, nitrogen IMS gas flow of 50 mL/h, both were critical parameters), and waves of either 25 V at 1000 m/s or 40 V at 1600 m/s (critical parameters). The IMS cell pressure was probed

using a Ceravac CTR 100 capacitance gauge (independent of the nature of the gas) interfaced to a CENTER TWO controller (Oerlikon Leybold Vacuum GmbH, Koeln, Germany). Mass spectra recorded with ion mobility parameters optimal for IMS resolution (Fig. S2I) reveal much more energetic conditions than on other instruments. Switching the instrument to the TOF mode restored the 3-NH<sub>4</sub> complex as the base peak. In IMS mode, lowering the helium cell gas flow increased the ammonium ion loss, whereas lowering the IMS nitrogen gas flow (and therefore lowering the IMS pressure) decreased the ammonium ion loss (Fig. S2J). This highlights that collisions before the IMS cell are a major source of ion heating in the IMS instruments [34,35], that nitrogen is causing more heating than helium, and that the higher IMS cell pressure at stake in the Synapt G2 compared to the Synapt G1 instrument results in more ion heating. Note that due to this higher pressure, a higher bias voltage must be used on the Synapt G2 (35 V) than on the Synapt G1 (8V) to optimize ion transmission.



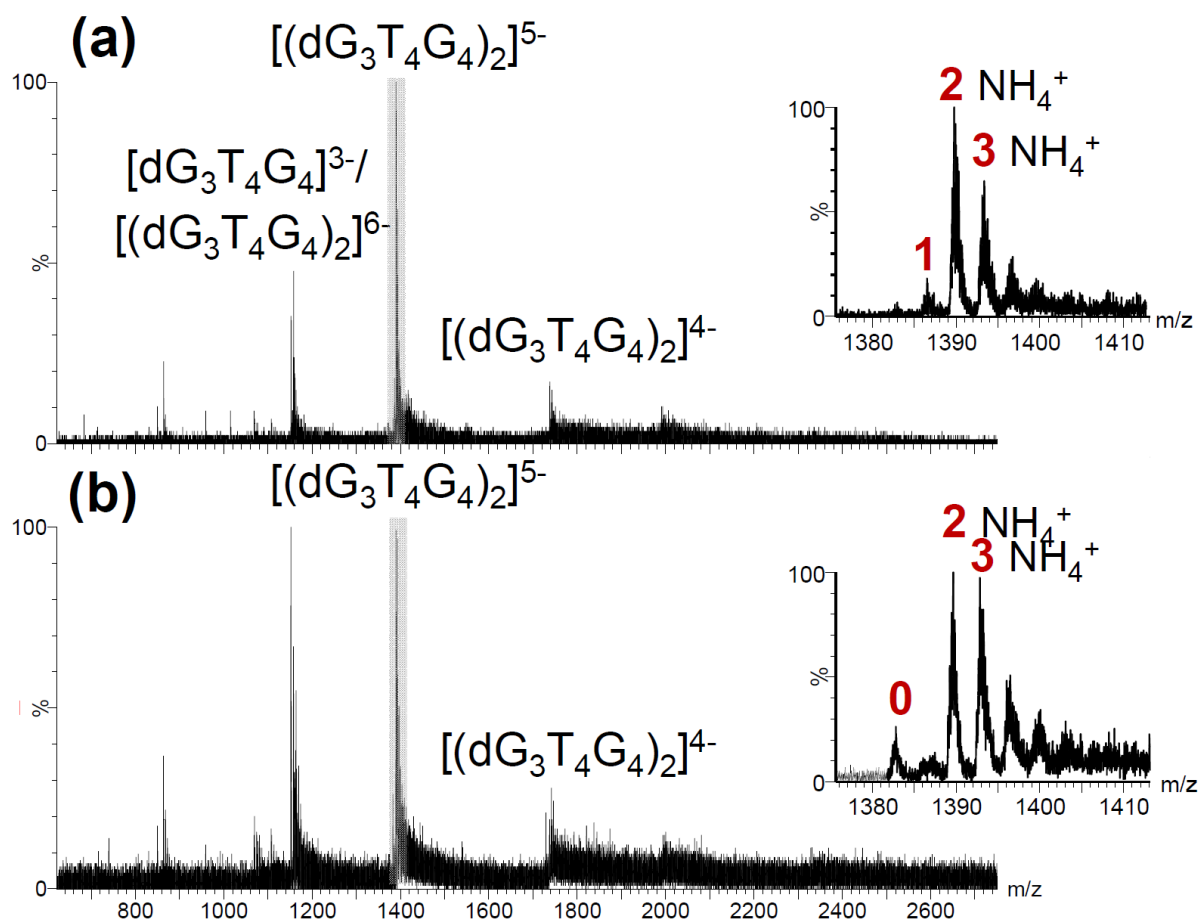
**Figure S2:** Influence of instrument choice and instrument tuning parameters on ammonium ion preservation in  $[(dG_4T_4G_4)_2]^{5-}$ . The number of preserved ammonium is written in red. **(A-D)** Solarix mass spectra: (A)  $T_{\text{dry gas}} = 20^\circ\text{C}$ , Skimmer1 = -5 V, Collision energy = 0 V; (B)  $T_{\text{dry gas}} = 20^\circ\text{C}$ , Skimmer1 = -40 V, Collision energy = 0 V; (C)  $T_{\text{dry gas}} = 20^\circ\text{C}$ , Skimmer1 = -5 V, Collision energy = 1.5 V; (D)  $T_{\text{dry gas}} = 60^\circ\text{C}$ , Skimmer1 = -5 V, Collision energy = 1.5 V. **(E-F)** Q-TOF Ultima Global mass spectra: (E)  $T_{\text{source}} = 60^\circ\text{C}$ ,  $T_{\text{desolv}} = 40^\circ\text{C}$ , Cone = 100 V, RFlens1 = 60 V; (F)  $T_{\text{source}} = T_{\text{desolv}} = 20^\circ\text{C}$ , Cone = 100 V, RFlens1 = 60 V; (G)  $T_{\text{source}} = T_{\text{desolv}} = 20^\circ\text{C}$ , Cone = 35 V, RFlens1 = 60 V; (H)  $T_{\text{source}} = T_{\text{desolv}} = 20^\circ\text{C}$ , Cone = 35 V, RFlens1 = 0 V. **(I-L)** Synapt G2 HDMS mass spectra (all spectra recorded with a bias of 35 V): (I)  $p_{\text{IMS}} = 2.760$  mbar (He Cell flow rate: 180 mL/h,  $\text{N}_2$  IMS flow rate: 125 mL/h), travelling waves: 40 V at 1000 m/s; (J)  $p_{\text{IMS}} = 1.820$  mbar (He cell flow rate: 180 mL/h,  $\text{N}_2$  IMS flow rate: 50 mL/h), travelling waves: 40 V at 1000 m/s; (I)  $p_{\text{IMS}} = 1.820$  mbar, travelling waves: 25 V at 1000 m/s; (I)  $p_{\text{IMS}} = 1.820$  mbar, travelling waves: 40 V at 1600 m/s.

**S3. Full scan mass spectra, and zooms on the ammonium ion distributions for the two lowest charge states**



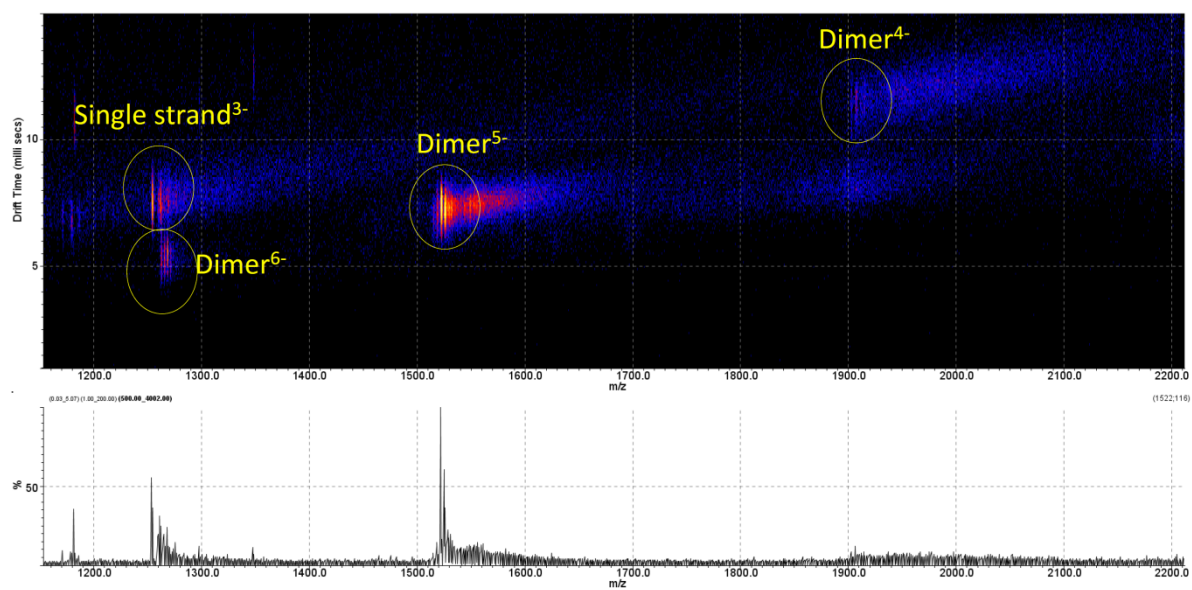
**Figure S3:** ESI-MS spectra in soft instrumental conditions on the Solarix ESI-FTICR instrument. Solution conditions: 100% aqueous 150 mM  $NH_4OAc$  with 10  $\mu M$  DNA strand (A)  $dG_4T_4G_4$ , (B)  $dG_4T_3G_4$ , (C)  $dG_3T_4G_4$  ( $T_{dry\ gas} = 150^\circ C$ ), (D)  $dG_4(T_4G_4)_3$  ( $T_{dry\ gas} = 220^\circ C$ ), (E)  $dG_2T_2G_2TGTG_2T_2G_2 = TBA$ . The spectra on the left are the full mass spectra, and the insets on the right are zooms on peaks shaded in gray (dimeric structures for A-C, intramolecular structures for D-E) with indication of the number of ammonium ions preserved.

**S4. (dG<sub>3</sub>T<sub>4</sub>G<sub>4</sub>)<sub>2</sub> takes up a third specific ammonium ion: Q-TOF and Synapt G1 results**



**Figure S4:** (a) Synapt G1 HDMS and (b) Q-TOF Ultima Global ESI mass spectra recorded on dG<sub>3</sub>T<sub>4</sub>G<sub>4</sub>. The G-quadruplex is known to capture two ammonium ions between its three G-quartets, but the mass spectra (see insets) reveal the presence of one additional specific ammonium ion binding site (see main text for discussion).

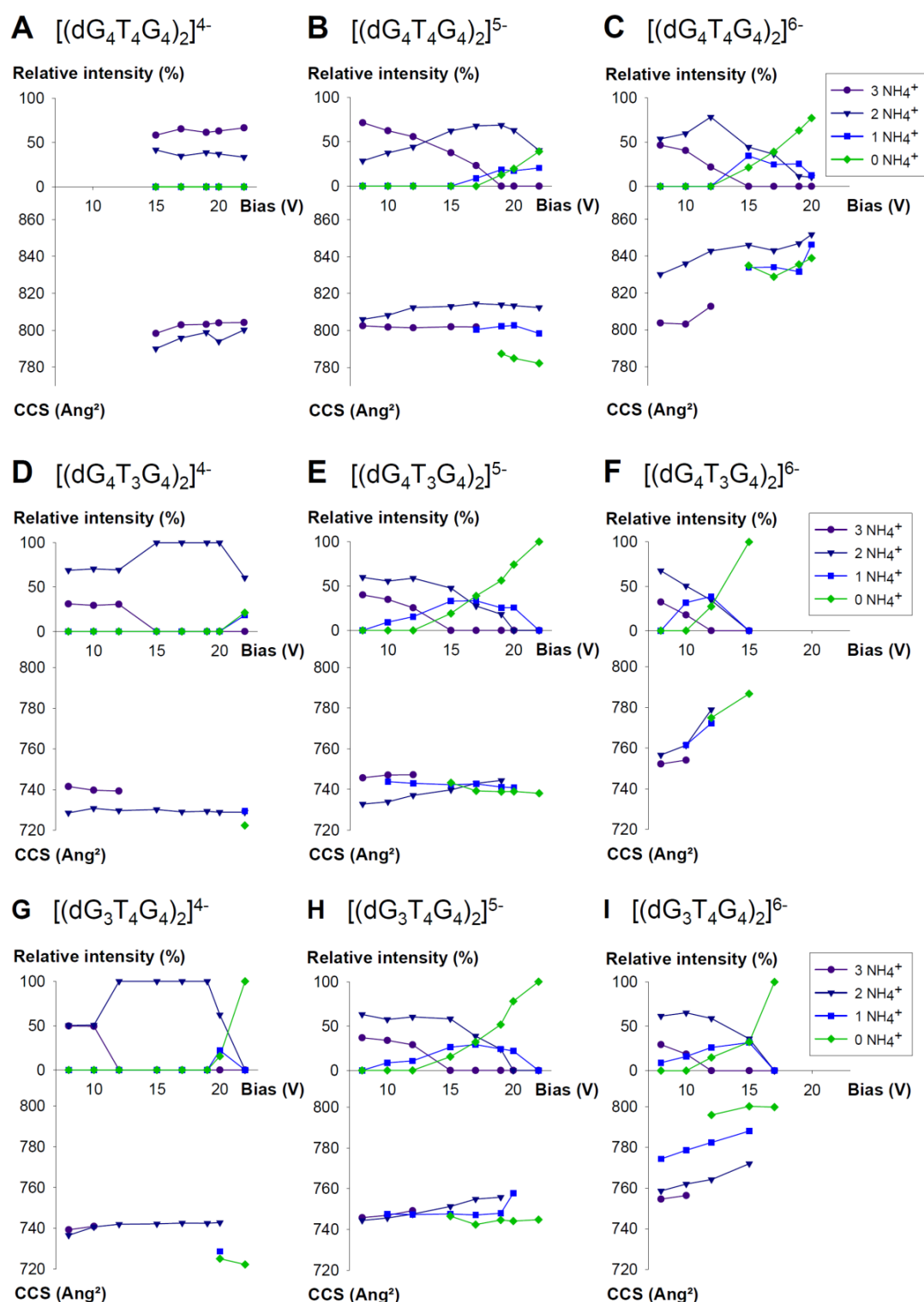
## S5. Representative driftscope spectrum



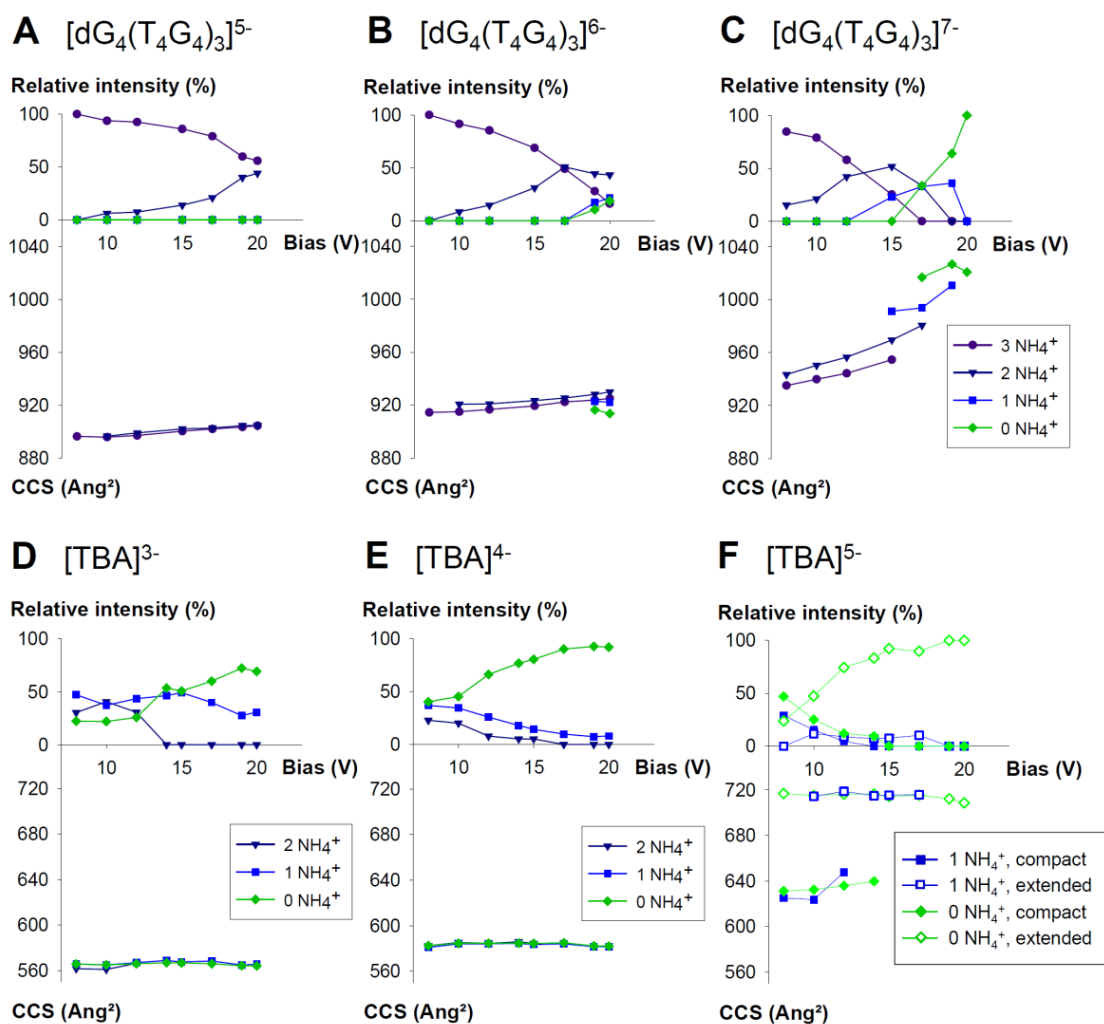
**Figure S5.** Driftscope spectrum obtained on the Synapt G1 HDMS with  $(dG_4T_4G_4)_2$  at a bias voltage of 15 V (the lowest limit for detection of the dimer<sup>4-</sup>).



## S6. Complete dataset of CCS as a function of the oligo, charge state, and bias voltage



**Figure S6-1:** Influence of the bias voltage and of the number of preserved ammonium ions on the collision cross sections (CCS) of the **bimolecular** G-quadruplexes at different charge states. The upper plot indicates the relative abundance of each species as a function of the bias.



**Figure S6-2:** Influence of the bias voltage and of the number of preserved ammonium ions on the collision cross sections (CCS) of the **intramolecular** G-quadruplexes at different charge states. The upper plot indicates the relative abundance of each species as a function of the bias. For  $[\text{TBA}]^{5-}$ , the collision cross section distribution showed two distinct peaks, defined as “compact” (lower CCS, filled symbols) and “extended” (higher CCS, open symbols). Note that for  $[\text{TBA}]^{5-}$ , both CCS peaks are present with and without ammonium ion bound, albeit in different proportions.

## S7. Supplementary references

1. Miller, M. C.; Buscaglia, R.; Chaires, J. B.; Lane, A. N.; Trent, J. O. Hydration Is a Major Determinant of the G-Quadruplex Stability and Conformation of the Human Telomere 3' Sequence of d(AG(3)(TTAG(3))(3)). *J. Am. Chem. Soc.* 2010.
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