

Estimating Collision Cross Sections of Negatively Charged *N*-Glycans using Travelling Wave Ion Mobility-Mass Spectrometry

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Supporting Information

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Abstract and Table of Content

The supporting information contains detailed information about the experimental parameter of the glycan CCS measurements:

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1 Absolute CCS Measurements

Measurement of absolute collision cross sections (CCS) were performed on a modified Synapt G1 HDMS (Waters, Manchester, UK) quadrupole-IM-MS instrument with a 18 cm long linear rf-confined drift tube which was described in detail previously.¹ IMS separation was followed by mass analysis using an orthogonal time-of-flight (ToF) mass analyzer. Typically, 5 μ L of sample were ionized using a nano-electrospray source (nESI) from platinum-palladium-coated borosilicate capillaries prepared in-house.² Settings for *N*-glycan and dextran measurements were: capillary voltage, 1.0-1.5 kV; sample cone 50 V, extractor cone 10 V; cone gas, 40 L/h; trap collision voltage, 10 V; trap DC bias, 25 V; IMS drift voltage, 50-150 V; backing pressure, 2-3 mbar; trap pressure, 5.2×10^2 mbar (He), 2.6×10^2 mbar (N₂); ion mobility gas, He or N₂; ion mobility cell pressure, 3.5 mbar (He), 0.87 mbar (N₂); time-of-flight analyzer pressure, $1.8-2.2 \times 10^6$ mbar. Data were processed using MassLynx software (Version 4.1, Waters, Manchester, UK) and OriginPro 8.5 (OriginLab Corporation, Northampton). In addition a self-written python script was used to extract the arrival time distributions (ATDs) of the relevant signals and to determine the drift times by fitting the ATDs with a Gauss distribution function.

CCS values were determined from the slopes of drift time versus reciprocal drift voltage plots as described previously. At constant temperature (*T*) and pressure (*p*), the velocity of the ions in

the IMS cell (v) is directly proportional to their mobility (K) and the applied electric field (E) (Eq. 1).

$$v = KE \quad (1)$$

The drift time t_D that is needed to traverse a cell of length L is proportional to the inverse mobility ($1/K$) as well as the inverse field ($1/E$) (see Eq. 2). Each sample was measured at eight different drift voltages ranging from 50 to 150 V at two drift gasses (He and N₂). By plotting the drift time t_D vs. the reciprocal drift voltage the mobility of an ion can be obtained from the slope of the linear regression (correlation coefficients typically above 0,9996). The intercept of the fit t_0 corresponds to the time required to transport the ions from the end of the drift region into the mass analyzer.

$$t_D = \frac{L}{KE} + t_0 \quad (2)$$

From the obtained mobilities, absolute CCSs were calculated using the Mason-Schamp equation:³

$$CCS = \frac{3e}{16N} \sqrt{\frac{2\pi}{\mu k_B T}} \frac{1}{K} \quad (3)$$

where N is the drift gas number density, μ the reduced mass of the ion and drift gas, k_B the Boltzmann constant and T the temperature. The reported CCSs represent averages of two replicates acquired in independent measurements.

2 TW IM-MS Measurements and CCS Estimation

Travelling wave (TW) IM-MS measurements were performed on a Synapt G2-S IM-MS instrument (Waters, Manchester, UK). The samples were diluted in water/methanol 1:1 (v:v) and

typically 5 μL were sprayed from a platinum-palladium-coated borosilicate capillaries prepared in-house.²

Typical instrument settings were: capillary voltage, 0.8-0-1.0 kV; sample cone 125 V; source offset 100 V, source temperature, 60°C; trap collision voltage, 50 V; trap collision voltage, 2 V; helium cell gas flow, 180; IMS gas flow, 90 mL/min; wave velocity, 500, 550, 600, 650, and 700 m/s; wave height, 40 V; backing pressure, 3.8 mbar; trap pressure, 2.3×10^2 mbar; ion mobility gas, N_2 ; ion mobility cell pressure, 3.4 mbar; time-of-flight analyzer pressure, 1.1×10^6 mbar. The instrument was mass-calibrated using a solution of caesium iodide (100 mg/mL) and the data were processed with MassLynx 4.1 software (Waters, Manchester, UK). ATDs of the relevant signals were manually extracted from raw data using DriftScope and MassLynx and fitted with a Gaussian distribution function using OriginPro.

CCS estimation was carried out as reported previously.^{1,4,5} Briefly, the measured drift times were corrected for m/z dependent delay time in the instrument with c being an empirically determined constant ($c = 0.001 \times \text{EDC delay coefficient}$).

$$t'_D = t_D - c \sqrt{m/z} \quad (4)$$

Absolute CCSs (CCS^{DT}) were corrected for charge and reduced mass.

$$\text{CCS}' = \frac{\text{CCS}^{\text{DT}}}{z} \sqrt{\frac{1}{m_{\text{ion}}} + \frac{1}{m_{\text{gas}}}} \quad (5)$$

The plot of $\ln(t'_D)$ against $\ln(\text{CCS}')$ can be fitted with a linear regression (Eq. 6), with x being the slope and $\ln A$ the intercept, and used as a calibration curve.

$$\ln(\text{CCS}') = x \ln(t'_D) + \ln A \quad (6)$$

When solved for CCS^{DT} with the addition of Eq. 5 estimated CCSs (CCS^{DT} now replaced with CCS^{EST}) can be obtained from drift times measured on TW instruments.

$$\text{CCS}^{\text{EST}} = A \cdot z \cdot t_D' \times \sqrt{\frac{1}{m_{\text{ion}}} + \frac{1}{m_{\text{gas}}}} \quad (7)$$

Absolute CCS^{DT} served as calibrants to estimate N_2 and pseudo He CCSs from TW IM-MS measurements in N_2 .

To estimate CCSs, each sample was measured at five wave velocities on a Synapt G2-S instrument. For every wave velocity one calibration curve was generated and the resulting five estimated CCSs of one ion were averaged with typical standard deviations of 1%.

References

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