Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

Collision cross sections of high-mannose N-glycans in commonly observed adduct states - Identification of gas-phase conformers unique to [M-H]- ions

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Abstract: Here we report collision cross sections (CCS) of high-mannose N-glycans as $[M+Na]^+$, $[M+K]^+$, $[M+H]^+$, $[M+Cl]^-$, $[M+H2PO4]^-$ and $[M-H]^-$ ions measured via drift tube (DT) ion mobility-mass spectrometry (IM-MS) in helium and nitrogen gases. Further analysis using traveling wave (TW) IM-MS revealed the existence of distinct conformers exclusive to $[M-H]^-$ ions.

Experimental Details

N-glycan sample preparation

Synthetically derived N-glycans (Dextra, Reading UK) were diluted with HPLC grade water to a final concentration of 150µM. 1µl from the stock solution was added to 8µl 1:1 MeOH/H_20 (HPLC grade) and 1µl 100mM solution of either KCl, $NH_4H_2PO_4$, ammonium acetate, NH_4Cl or 0.1% formic acid to promote adduct formation. Porcine thyroglobulin, bovine RNAse B and chicken ovalbumin were purchased from Sigma Chemical Co. Ltd. (Poole, Dorset, UK) and N-glycans were released chemically by hydrazinolysis and subsequently re-N-acetylated. Sample solutions were stored at -20°C until IM-MS analysis.

DT IM-MS and absolute CCS measurements

Absolute collision cross sections (^{DT}CCS) measurements were performed with a modified Synapt G1 HDMS (Waters, Manchester, UK) hybrid quadrupole IM-MS instrument modified with a linear drift tube as previously described[1]. For each analysis 2µl of sample was ionized by nano-electrospray ionization (nano-ESI) from gold-coated borosilicate glass capillaries made in-house[2]. Instrument setting for negative ion analysis were as follows: capillary voltage, 0.8-1kV; sample cone, 150V; extraction cone, 10V; cone gas, 40L/h; trap collision voltage, 10V; trap DC bias, 25V; IMS drift voltage, 50-150V; ion mobility cell pressure, 3.3 mbar (He), 1.2 mbar (N_2). Instrument setting for positive ion analysis were as follows: capillary voltage, 1-1.5kV; sample cone, 80V; extraction cone, 10V; cone gas, 40L/h; trap collision voltage, 10V; trap DC bias, 22V; IMS drift voltage, 50-150V; ion mobility cell pressure, 3.3 mbar (He), 1.1 mbar(N_2). Data was processed with MassLynx v4.1 and Driftscope (Waters, Manchester, UK). Experimental arrival time distributions (ATDs) were fit to a Gaussian distribution to determine the drift time. ^{DT}CCS were calculated from the slopes of the drift time versus reciprocal drift voltage (50, 55, 60, 70, 80, 100, 125 and 150V) plots as previously described [1, 3]. Briefly, the velocity of ions through the IMS cell is proportionate to the mobility (K) and applied electric field (E) at constant pressure (p) and temperature (T) (Equation 1).

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

The drift time (t_D) required to traverse the IM cell of length L is proportional to the inverse mobility ($1/K$) and the inverse field ($1/\mathrm{E}$) (Equation 2). The intercept from a linear regression of drift time (t_D) versus the inverse drift voltage applied for each acquisition gives t_0, which represents the time ions spend from the end of the IMS cell to the mass detector from which mobility (K) can be determined:

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

CCS values were then calculated using the Mason-Schamp equation[4]:

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

Where e is the ion charge, N is the drift gas number density, μ is the reduced mass of the ion and drift gas, k_B is the Bolzmann constant and T is the temperature measured in the IMS cell.

TW IM-MS measurements

Traveling wave (TW) IM-MS measurements were performed on a Synapt G2Si instrument (Waters, Manchester, UK). For each analysis 2µl of sample was ionized by nano-electrospray ionization (nano-ESI) from

gold-coated borosilicate glass capillaries made in-house[2]. Instrument setting for negative ion analysis were as follows: capillary voltage, 0.8-1kV; sample cone, 100V; extraction cone, 25V; cone gas, 40L/h; source temperature, 80°C; trap collision voltage, 4V; transfer collision voltage, 4-100V; trap DC bias, 45V; wave velocity, 650 m/s; wave height, 40 V; trap gas flow, 2ml/min; IMS gas flow, 80 ml/min. Data was acquired and processed with MassLynx v4.1 and Driftscope software (Waters, Manchester, UK). Arrival time distributions (ATDs) were fit to a single or double Gaussian distribution prior to estimating experimental ^{TW}CCSs. A dextran calibration ladder with known ^{DT}CCS was for estimating N-glycan ^{TW}CCS values as previously described[3, 5]. Briefly, measured drift times (t_D) were corrected for *m/z* dependent delay time from Equation 4 where c is an empirically determined constant (c = 0.001 x EDC (enhanced duty cycle) delay coefficient).

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

Absolute dextran ^{DT}CCS were corrected for charge and reduced mass (Equation 5).

$$CCS' = CCS^{DT}/[z \times \left(\frac{1}{\mu}\right)^{\frac{1}{2}}] \qquad (5)$$

A linear correlation plot of ln(CCS') versus ln(dt') (R^2>0.99) gives two constants termed the fit-determined constant A and the 'exponential factor' X from the equation:

$$ln\, CCS' = X \times ln\, t_D + \ln A \qquad (6)$$

Experimental ATDs were converted to ^{TW}CCS values from Equation 5.

Supplementary Tables and Figures

Table S1. Estimated ^{TW}CCS values of all investigated N-glycans.

	Glycan	Collision Cross Section (Å²)					
		Negative Ions			Positive Ions		
		M-H	M+Cl	M+H_2PO_4	M+H	M+Na	M+K
Synthetic (Dextra)	Man3	266.9	277.3	279.4	301.1	304.6	295.3
	Man5	318.4/329.5	331.5	337.3	346.6	351.9	356.1
	Man6	346.0/356.8	355.4	362.4	370.1	372.5	368.6
	Man7	376.0	378.1	385.4	391.9	393.9	398.5
	Man8	406.2	413.0	415.7	421.4	422.8	423.2
	Man9	416.7/432.7	433.6	437.6	443.6	445.0	447.7
	Man9Glc	429.9/445.1	452.8	451.6	458.8	461.8	464.0
thyroglobulin	Man5	320.5/331.3	333.4	338.7			
	Man6	347.6/358.2	357.6	364.8			
	Man7	379.9	382.1	387.8			
	Man8	406.8	413.1	413.5			
	Man9	419.9/436.1	436.6	435.1			
ovalbumin	Man3	270.2	277.9	280.8			
	Man5	321.8/332.5	334.8	339.2			
	Man6	348.6/359.2	358.5	365.2			
RNase B	Man5	319.4/330.0	333.1	337.5			
	Man6	348.3/358.7	357.9	363.9			
	Man7	380.8	382.3	387.7			
	Man8	408.2	415.4	415.3			

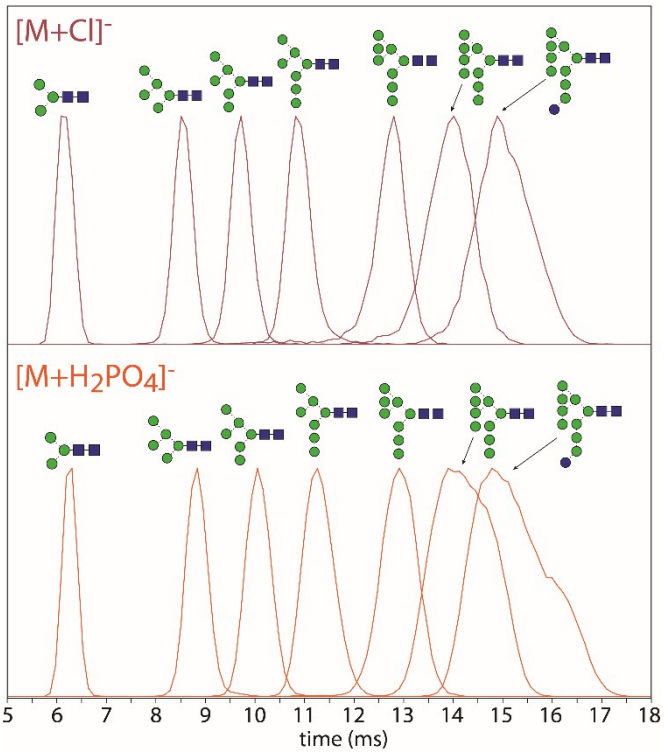

Figure S1: Arrival time distributions of synthetic high-mannose N-glycans as $[M+Cl]^-$ and $[M+H_2PO_4]^-$ ions.

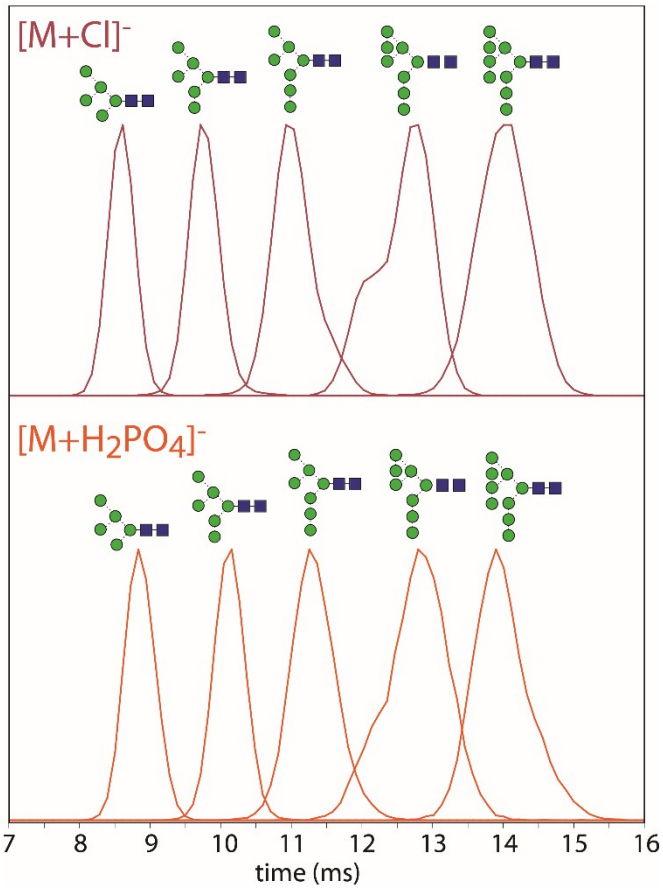

Figure S2: Arrival time distributions of thyroglobulin high-mannose N-glycans as $[M+Cl]^-$ and $[M+H_2PO_4]^-$ ions.

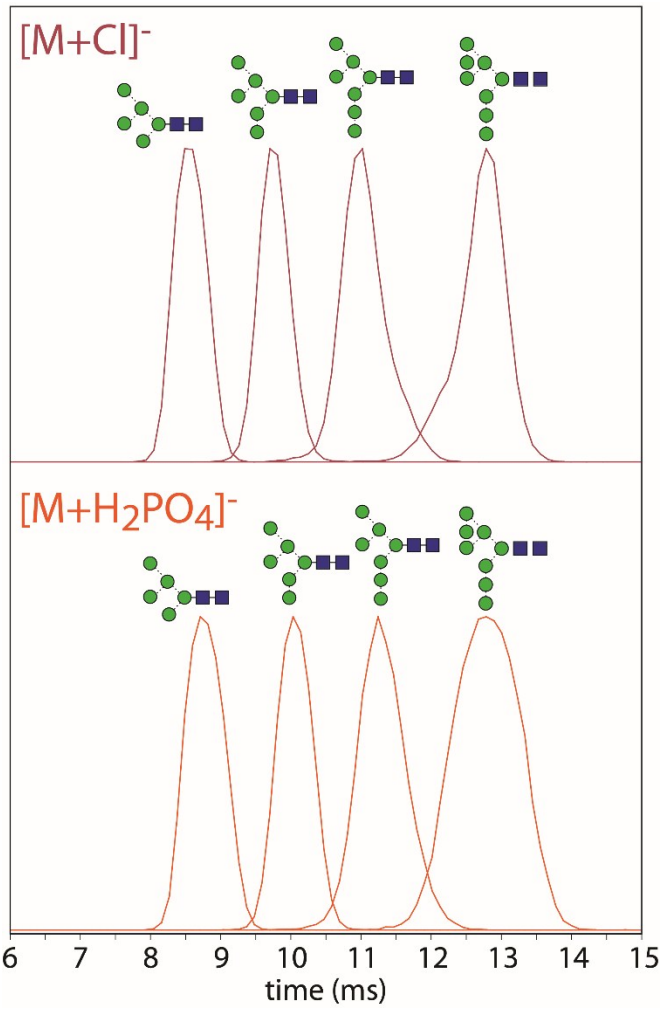

Figure S3: Arrival time distributions of RNase B high-mannose N-glycans as $[M+Cl]^-$ and $[M+H_2PO_4]^-$ ions.

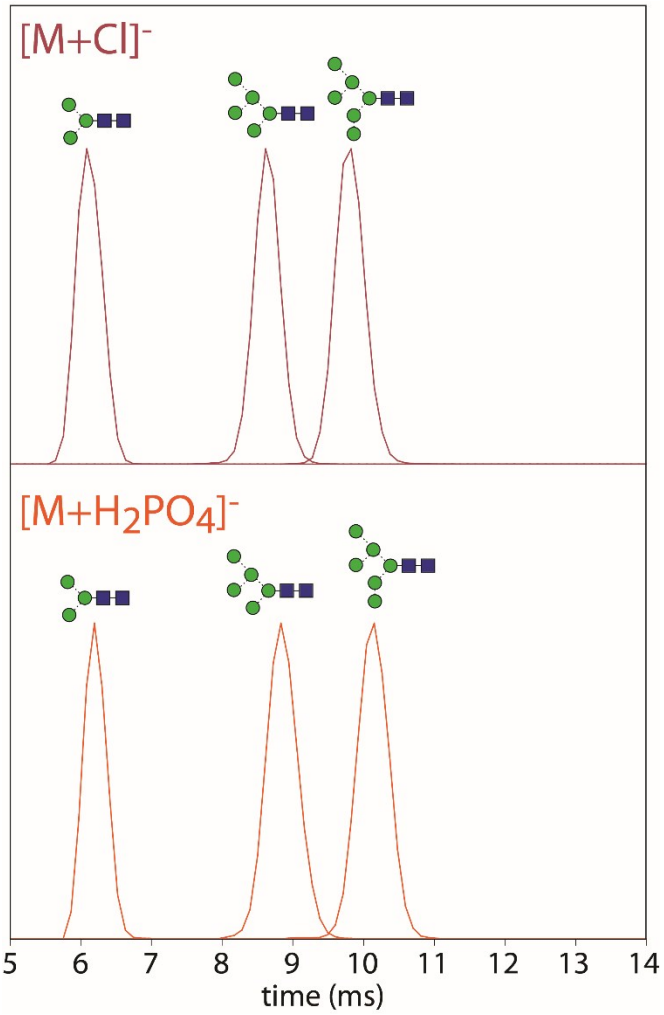

Figure S4: Arrival time distributions of ovalbumin high-mannose N-glycans as $[M+Cl]^-$ and $[M+H_2PO_4]^-$ ions.

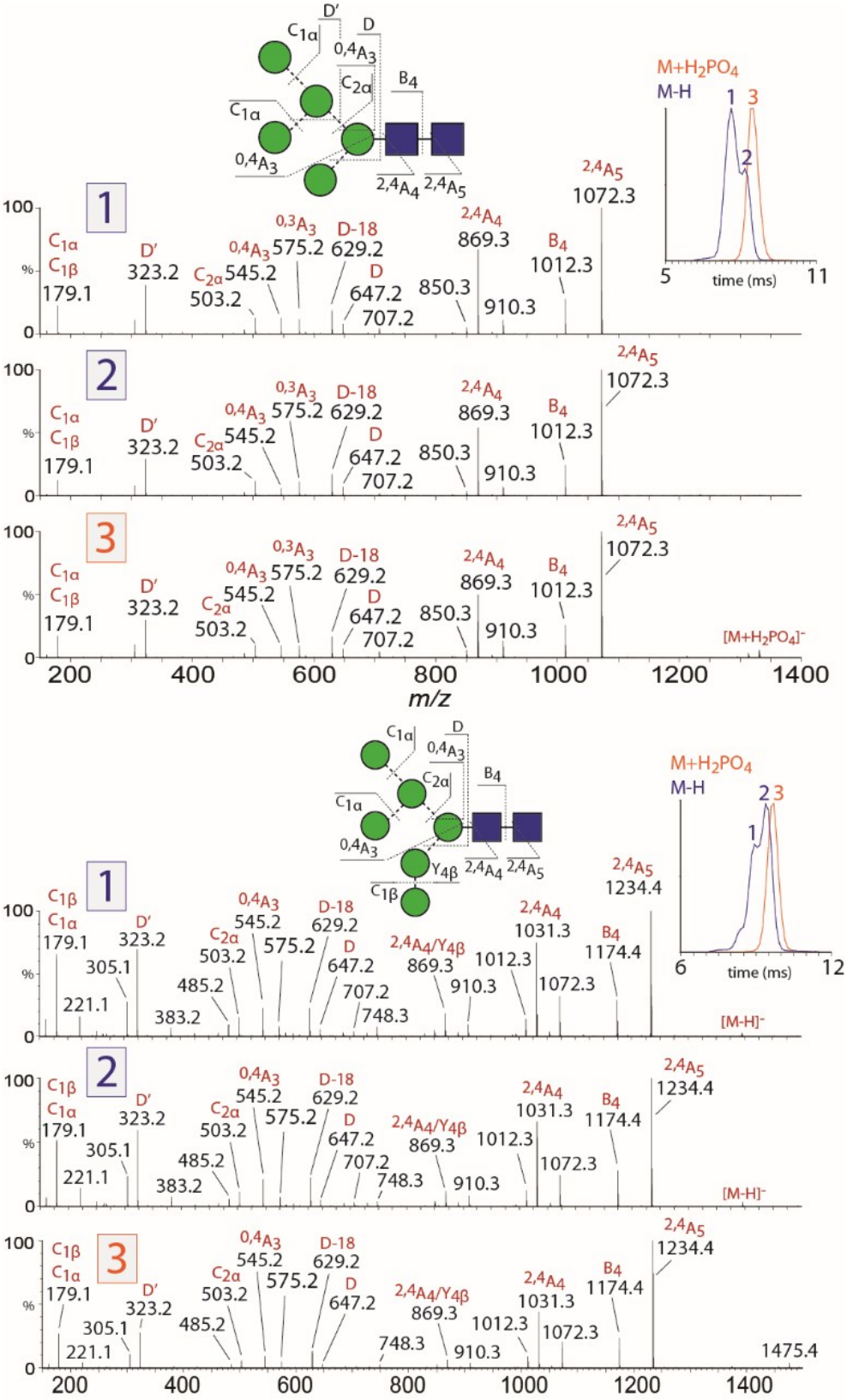

Figure S5: MS/MS spectra of Man5 and Man6 $[M-H]^-$ and $[M+H_2PO_4]^-$ ions from thyroglobulin. $[M-H]^-$ spectra were summed from peak 1 or peak 2. Fragmentation of $[M-H]^-$ and $[M+H_2PO_4]^-$ ions gave identical spectra and corresponds to the same structures for both Man5 and Man6.

References

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