Supplementary Information

OrbiSIMS spatially resolves isomeric molecules on surfaces

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Supplementary Note 1: Experimental methods

OrbiSIMS silver measurements: An instrument built-in silver reference sample was used. Datasets were acquired using an OrbiSIMS instrument (Hybrid SIMS, IONTOF GmbH) in single beam depth profiling mode using a 30 keV Bi_3^+ ion beam from a liquid metal ion source with an average primary ion beam current of 2 nA in a cycle time of 200 µs. The beam was set to scan an area of 342 µm × 342 µm of which positively charged secondary ions were collected from the central 300 µm × 300 µm of the crater. 70 x 70 pixels were used per scan, which resulted in a single injection per scan. The analyser extraction voltage was set at 2500 V, 2000 V and 500 V (data in main text corresponds to 2000 V). The Orbitrap mass analyser was operated with 512 ms injection time and 512 ms transient time for a mass resolving power of 240,000 at *m/z* 200. For scanning the V_T, the He collision cell pressure was kept at 0.12 mbar in high collisional cooling mode and the target potential was varied automatically during acquisition. Details of method are described elsewhere.[1] Silver clusters were used to calibrate the Orbitrap *m/z* scale in both positive and negative polarities.

Cytochrome C sample preparation: Cytochrome C from equine heart (purchased from Sigma-Aldrich) was dissolved at a concentration of 100 µM in a 1:1 (v/v) mixture of water and methanol containing 0.1% (v/v) formic acid. A 1 µL aliquot of the solution was manually deposited onto a silicon wafer using a micropipette. The droplet was allowed to air dry, forming a spot approximately 1–2 mm in diameter.

OrbiSIMS cytochrome C measurements: Datasets were acquired using an OrbiSIMS instrument (Hybrid SIMS, IONTOF GmbH). Depth profiles were obtained in a single beam mode using a 20 keV Ar_{2300}^+ ion beam from a gas cluster ion source. The secondary ions extraction voltage was set at 2000 V. The average primary ion beam current was 30 pA in a cycle time of 200 µs. For each measurement, the beam was set to scan areas of 350 µm × 350 µm of which negatively charged secondary ions were collected from the central 300 µm × 300 µm of the crater. 70 x 70 pixels were used per scan, which resulted in a single injection per scan. Analysis of secondary ions using the Orbitrap mass analyser with 980 ms injection time, 512 ms transient time for a mass resolving power of 240,000 at *m/z* 200 and an *m/z* range between 150-2250. For V_T scans, the He collision cell pressure was kept at 0.13 mbar in high collisional cooling mode and the target potential was varied automatically during acquisition.

Trisaccharides samples preparation: Substrate Si wafers (SiO2/Si) were prepared by sonication in IPA (isopropyl alcohol) followed by a wipe cleaning using Kimtech paper. The final step of the cleaning was performed using a UV ozone cleaner model T10X10/OES/E from UVOCS INC. Analytical standards of raffinose, maltotriose and melezitose were bought from Sigma Aldrich / Merck (product IDs 95068, 07304 and 851493). Each powder was dissolved in DI water (10 mM) and spin-coated using a programmable spin coater from Laurel technologies corporation model WS-

400/500B lite series. Spin-coating parameters for the trisaccharide solution was set at 1500 RPM for a duration of 3 minutes onto UV-ozone cleaned silicon wafer substrates using 100 µL of the solution. For mixtures, 1:1 and 1:2 Raffinose:Maltotriose in solution, the same deposition method was applied.

OrbiSIMS trisaccharides measurements: Datasets were acquired using an OrbiSIMS instrument (Hybrid SIMS, IONTOF GmbH). Depth profiles were obtained in a single beam mode using a 20 keV Ar_{2800}^+ ion beam from a gas cluster ion source. The secondary ions extraction voltage was set at 2000 V. The average primary ion beam current was 24 pA in a cycle time of 200 µs (trisaccharides). For each measurement, the beam was set to scan areas of 543 µm × 543 µm of which negatively charged secondary ions were collected from the central 500 µm × 500 µm of the crater. 70 x 70 pixels were used per scan, which resulted in a single injection per scan. Analysis of secondary ions using the Orbitrap mass analyser with 512 ms injection time, 512 ms transient time for a mass resolving power of 240,000 at *m/z* 200 and an *m/z* range between 100-1500. For V_T scans, the He collision cell pressure was kept at 0.04 mbar in low collisional cooling mode and the target potential was varied automatically during acquisition. For P_{He} scans, V_T was fixed and the collision cell pressure was varied continuously. Details of the method are described elsewhere.[1] For the imaging experiments, a 20 keV Ar_{3000}^+ with a spot size of approximately 5 µm was used. The beam current was set at 26 pA with a duty cycle of 31.2 % of a cycle time of 100 µs. Each image was acquired over areas of 400 x 400 μm^2 with pixel sizes of 4 µm. The Orbitrap analyser settings were kept the same.

IMS Sample preparation: Each trisaccharide powder sample was dissolved in CH_3CN:H2O 9:1 (v/v) containing 0.1%v/v ammonia to 0.1 µmol/mL.

IMS Experiment: Each sample solution was directly infused into Synapt G2-Si HDMS instrument with Travelling Wave Ion Mobility Spectrometry (TWIMS) function (Waters, UK) through a chip-based nano-electrospray source (TriVersa NanoMate, Advion, US). The cone voltage and source temperature were set to 80 V and 100 °C respectively. The nitrogen drift gas flow was set at 90 mL/min for all TWIMS experiments. The traveling wave speed was set to 900 m/s and the wave height set at 40 V. Mass spectra and ion mobility spectra were processed using MassLynx V4.1 (Waters, UK).

Supplementary Note 2: Secondary ion dissociation model

Considering that the instrument is optimised to transmit secondary ions of mass, m_1 and charge z, with mean optimal kinetic energy K_{O1}, determined by their energy of formation K_f, the target potential V_{T1} and extraction potential V_E:

$$K_{O1} = \frac{m_1 v_1^2}{2} = z\,(V_{T1} + V_E) + K_f \tag{1}$$

considering that $z\,(V_{T1} + V_E) \gg K_f$ and that ions decay after extraction and I in a field free region (and preservation of velocity as a first order approximation), there could be another target potential, V_{T2}, that is optimal to transmit daughter ions of mass *m*$_2$ and kinetic energy K_2:

$$K_2 = \frac{m_2 v_1^2}{2} = \frac{m_2}{m_1} K_{O1} = z\,(V_{T2} + V_E) \tag{2}$$

Dividing **Equation 2** by **Equation 1**,

$$\frac{m_2}{m_1} = \frac{(V_{T2} + V_E)}{(V_{T1} + V_E)} \tag{3}$$

Which can be used to predict the position of maxima in V_T scans:

$$V_{T2} = (V_{T1} + V_E)\,\frac{m_2}{m_1} - V_E \tag{4}$$

For a conducting sample, V_{T1} is the optimised potential of 57.5 V and for silver clusters, there is a finite, discrete number of possible decays that follow the rule:

$$m_1 = m_2 + \mathit{\Delta}$$

$$\mathit{\Delta} = n_1 m_{Ag107} + n_2 m_{Ag109}$$

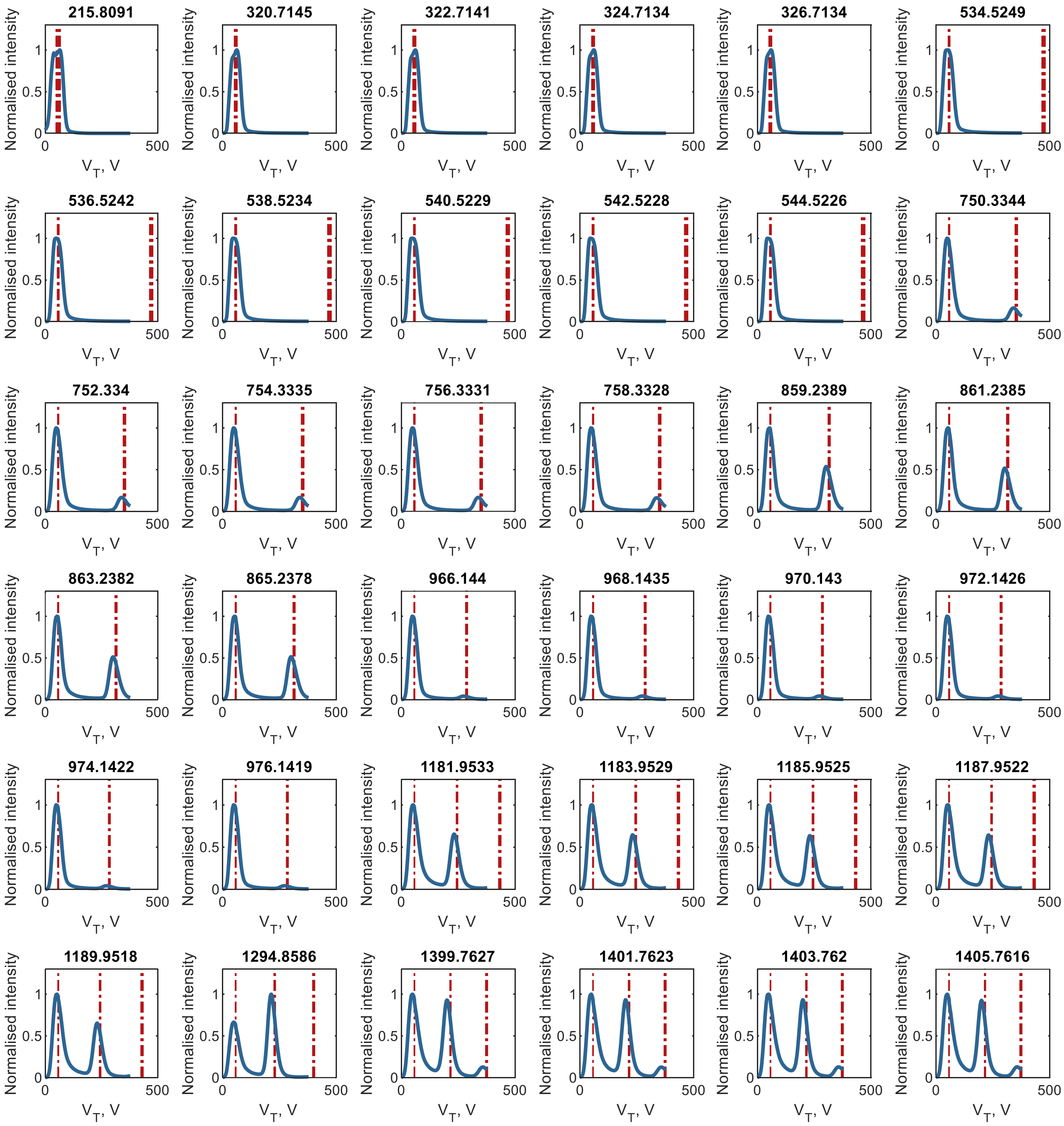
$$n_1, n_2 = 0, 1, 2, 3, 4, \dots$$

Therefore,

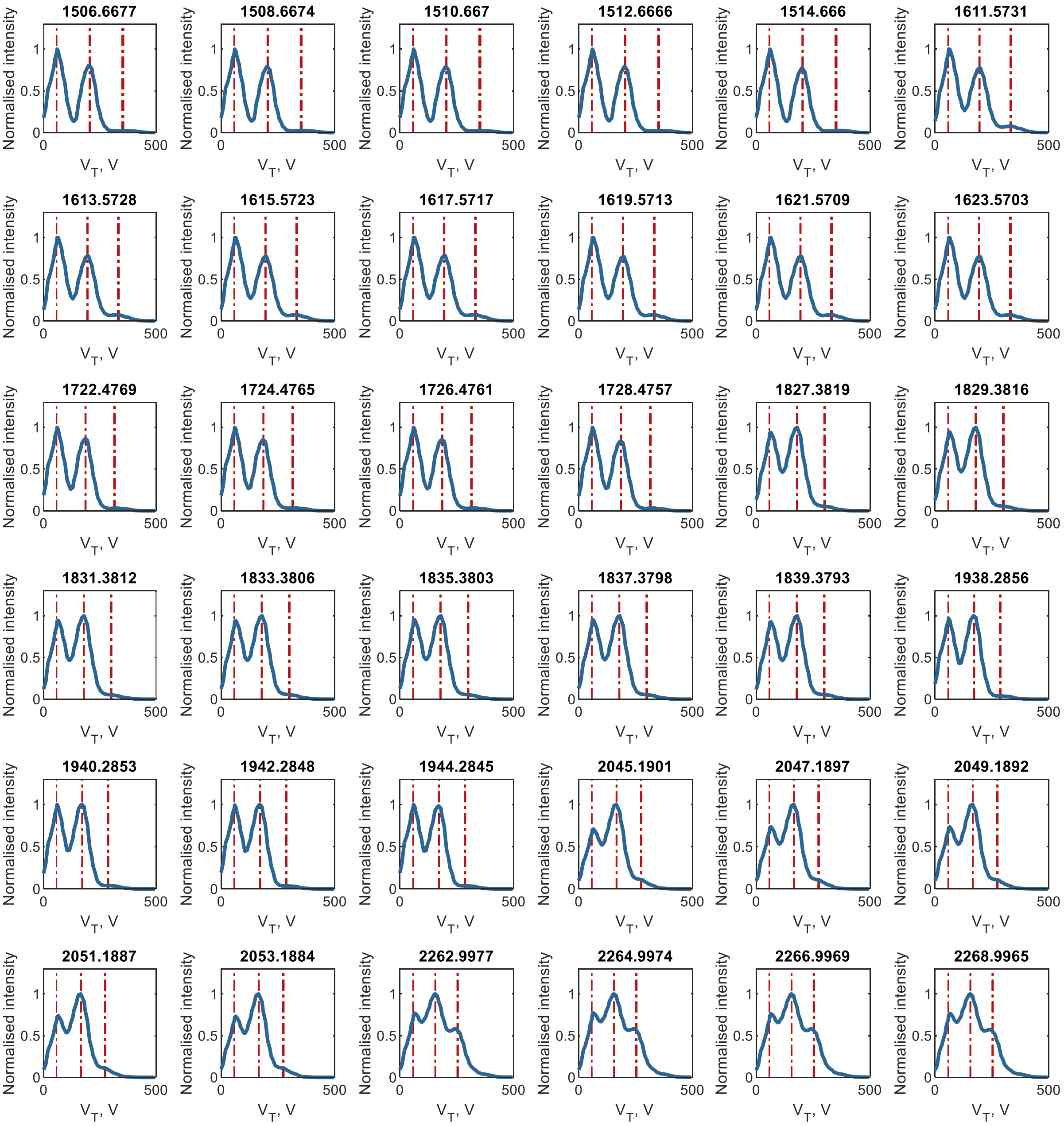
$$V_{T2} = (V_{T1} + V_E)\,\frac{m_2}{(m_2 + \mathit{\Delta})} - V_E \tag{5}$$

Where m_{Ag107} and m_{Ag109} are the masses of the two stable silver isotopes and n_1 and n_2 are either zero or positive integers. **Equation 5** can be used to predict all the maxima in the V_T spectra of silver clusters and the prediction shows great agreement with experimental data. (Supplementary **Figures 2.1 and 2.2)**. The OrbiSIMS instrument can only increase the target voltage up to 500 V but the model is able to show all other maxima that would occur if higher voltages were applied (**Supplementary Figure 2.3**).

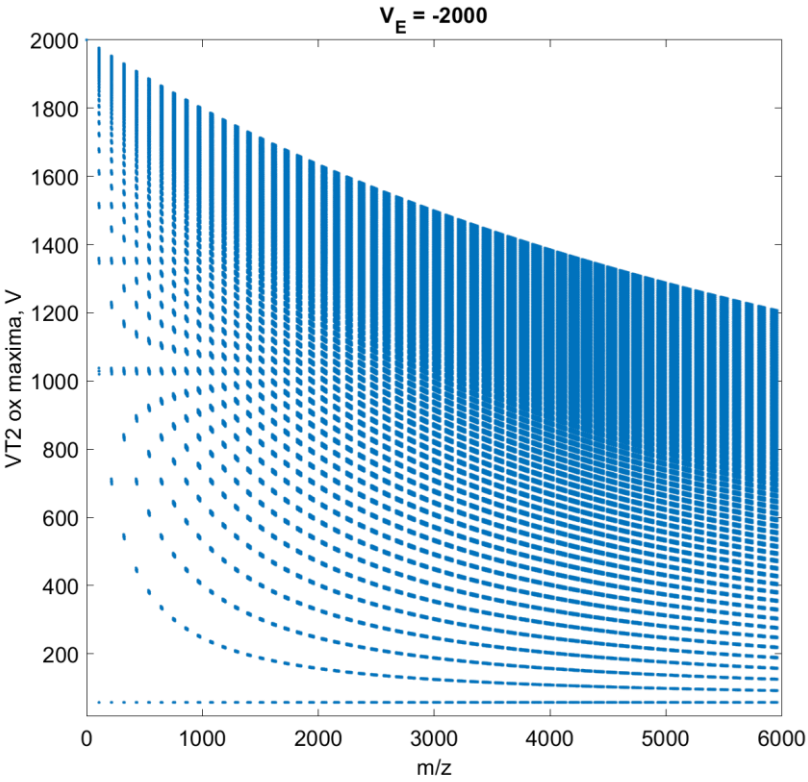
For the heme C molecule in the cytochrome C sample, V_{T1} is the position of the first maxima in a given V_T spectrum (which is not necessarily 57.5 V due to charge build-up on the sample's surface) and Δ will be a combination of multiples of the masses of S, HS, CH, CH_2, CH_3 and COOH (relating to dissociation of side chains). **Equation 5** can then be used to predict the position of maxima transmission in a V_T spectra for voltages up to and higher than 500 V, which shows a much more complex structure (**Supplementary Figure 2.4**)



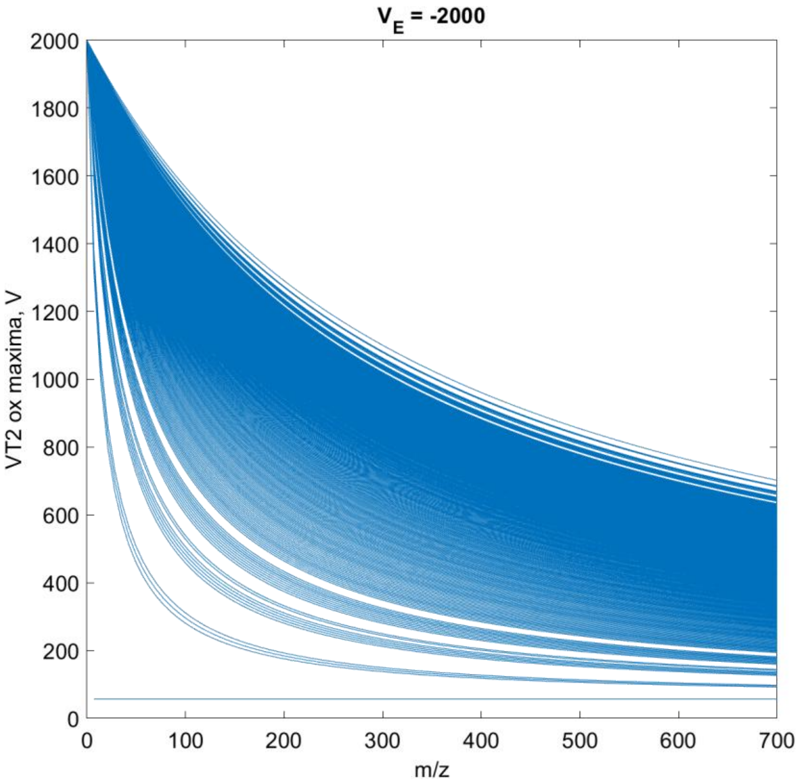
Supplementary Figure 2.1: V_T spectra for silver clusters with sizes 2 to 13 and mean intensity higher than 3000. Dashed lines represent predicted maxima using dissociation model.



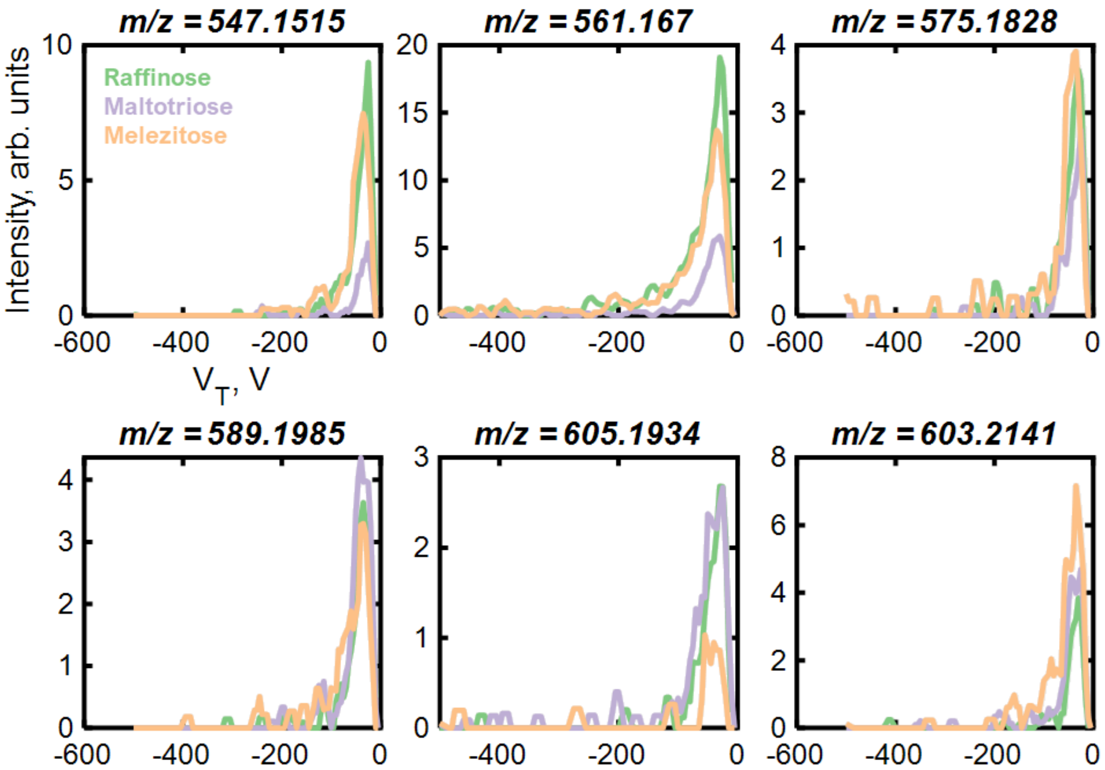
Supplementary Figure 2.2: V_T spectra for silver clusters with sizes 14 to 33 and mean intensity higher than 1700. Dashed lines represent predicted maxima using dissociation model.



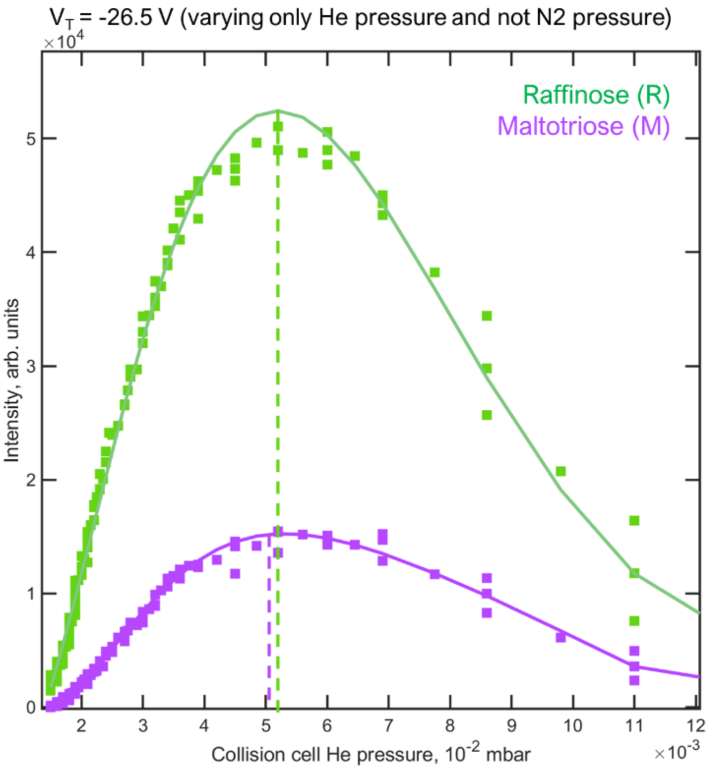
Supplementary Figure 2.3: Predicted values of V_T of maxima transmission as a function of detected *m/z* for silver clusters (using **Equation 5**).



Supplementary Figure 2.4: Predicted values of V_T of maxima transmission as a function of detected *m/z* for the heme C molecule in cytochrome C (using **Equation 5**).



Supplementary Figure 2.5: V_T spectra of the identified potential parent ions that dissociate to $[M\text{-}H]^-$.



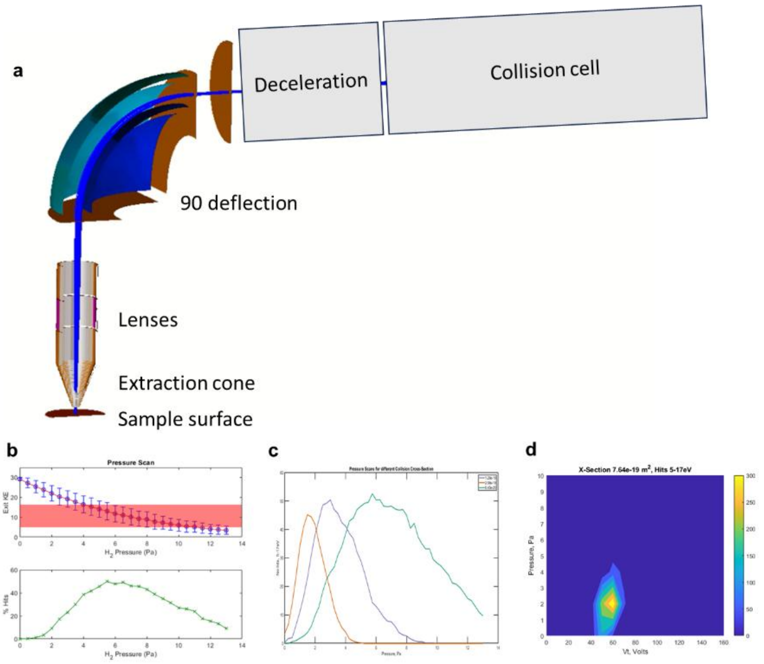
Supplementary Figure 2.6: Repeat experiment of He pressure scans of Raffinose and Maltotriose where the bent flatapole N_2 pressure was kept fixed. This confirms that the He pressure rules the behaviour observed and described in the main text.

Supplementary Note 3: Details of ion optics simulation framework

The ion optics model was made using SIMION (www.simion.com). The model contains a biased sample holder, an extraction cone, one set of einzel lenses for focusing of secondary ions and a hemispherical analyser (SI switch) to deflect the secondary ions by 90 degrees into the transfer system to the Orbitrap (Main text **Figure 1a**). With this set up it is possible to simulate V_T scans for secondary ions of arbitrary kinetic energy of formation, angular distribution and mass spectrum. We have also incorporated a user program to simulate decays and, by comparing against experimental data, the simulation framework may help to determine where the decays may occur within the system. To benchmark simulations against experiments, we simulated a V_T scan of Ag_{13}^+ ions that can decay to Ag_{12}^+ and subsequently to Ag_{11}^+ (Main text **Figure 1e**). For this simulation, dissociation only occurs between the exit of the einzel lens the entrance to the 90 degrees deflection unit, which is a field-free region and thus satisfies the hypothesis of Equation 1. The extraction potential was set at -2000 V and the deflection unit potential difference was set at 720 V. The initial spatial and kinetic energy distributions of the Ag_{13}^+ ions and probabilities of decay were set arbitrarily.

Description of SIMION workbench: The virtual SIMION workbench was created and developed with the aim of: i) Investigating the effect of cluster decay in a field-free region before the ions enter the SI switch, and comparing to experimental results; ii) modelling a gas collision cell similar to that found in the OrbiSIMS transfer, in order to investigate the effect of ion collision cross-section on the intensity detected and iii) progressing towards a long-term goal of a "digital twin" of the transfer process, which could be used to further investigate theories about phenomena that may be taking place. The workbench contains numerous components, referred to as "PA (Potential Array) Instances"; these are self-contained volumes inside which SIMION calculates the potential at all non-electrode points. These are then imported into the workbench. This is preferable to solving for the entire apparatus in one go, which is computationally intensive and requires large amounts of memory. To alleviate the issue of any discrepancies at the boundary between instances, shielding was added between the components. The ions were produced on a surface whose potential was at the Target Potential V_T, which could be set by the user. Their kinetic energy upon creation usually normally distributed around a mean of 50 eV with FWHM 10 eV (although this was varied for some tests). They then passed through an extraction cone, which took them to a "base" potential of -2000 V. Immediately behind this was placed an Einzel lens, to reduce the ion beam divergence as it passed towards the 90° SI switch. All of these components were part of the same "PA instance". The ion decay was implemented in a field-free region between the extraction cone / lens and the SI switch, using the *simion.experimental.add_particles* functionality introduced in SIMION 8.1.

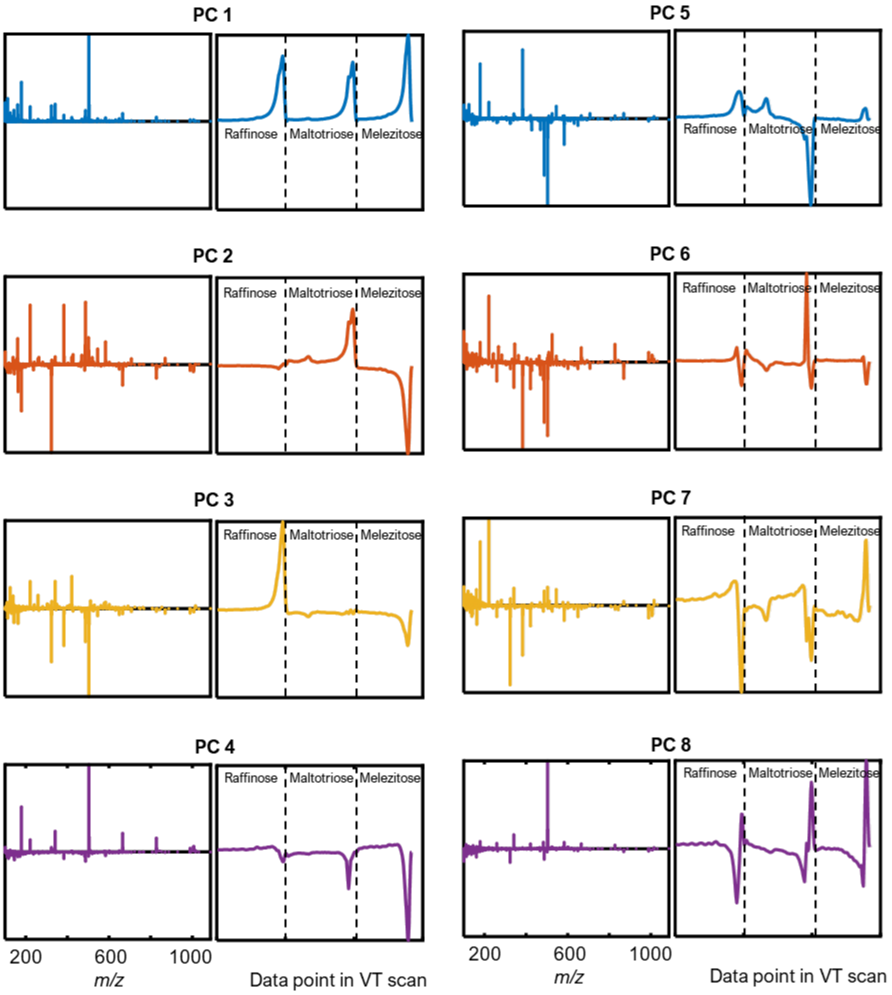
Tests were conducted using groups of $[Ag_{13}]^+$ clusters, which were permitted to decay up to two times, losing a silver atom each time (which were not modelled). Upon creation, the new ions were given the same velocities as their parents. This produced three distinct beams which, due to their differences in kinetic energy, were separated in the SI switch, by a potential difference of 720 V (although in later versions of the workbench this was altered to 746 V, which introduced improved shielding among other amendments). The potentials of the inner and outer surfaces of the SI switch were set symmetrically around -2000 V to deflect the beam through an angle of 90° and adjusted to give optimum transmission of the undecayed cluster ions at $V_T = 57.5$ V. Using these settings, the once-decayed and twice-decayed ions saw optimum transmission at or around $V_T = 230$ V and 440 V respectively, in agreement with theory. In earlier version of the workbench, after passing through the hemispherical analyser, the ions collided with a detector plate. The ability to run a VT scan was implemented via a “user program”. The workbench was extended to provide the functionality of a VT/P scan. After leaving the hemispherical analyser, the ions passed through a set of decelerating steps, followed by a collision cell. The collision cell contains a stationary, neutral background gas (He). The background gas simulation implemented a hard-sphere collision model available in the SIMION example documentation. In a similar manner to the potential of the biased sample holder, the gas pressure could be varied with a specified start point, end point, and increment value (**Supplementary Figure 3.1**).



Supplementary Figure 3.1: a Extended workbench functionality to produce V_T spectra coupled with pressure scans. **b** Exit kinetic energy of test ions as function of pressure in cooling cell (top) and number of effectively transmitted ions (within red band) as function of pressure in cooling cell (bottom). **c** Pressure scans for ions with same *m/z* but different collision cross sections. **d** A heat map showing number of effectively transmitted ions as a function of V_T and P.

Supplementary Note 4: Details of PCA analysis to find trends within V_T spectra

Principal component analysis (PCA) of the V_T spectra datasets was carried out using secondary ion masses as the variables and V_T values as observations. For each dataset, Surface Lab 7.3 (IONTOF GmbH) was used to perform an automated peak search on the total spectra for all three samples (raffinose, maltotriose and melezitose). Peak intensities were then exported for each observation. PCA was performed using the simsMVA software.[2] Data from V_T spectra for each isomer were arranged in a matrix combining all observations for each type of device for consistent factorisation.[3] Prior to PCA, data were scaled to account for non-uniform noise across the mass spectra.[4]



Supplementary Figure 4.1: PCA results of dataset combining V_T spectra of all three isomers raffinose, maltotriose and melezitose. For each PC, the left-hand side panel shows the loadings and the right-hand side panel shows the scores per data point in their V_T spectra.

References

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2. Trindade, G. F., Abel, M. & Watts, J. F. simsMVA : A tool for multivariate analysis of ToF- SIMS datasets. *Chemom. Intell. Lab. Syst.* (2018) doi:10.1016/j.chemolab.2018.10.001.

3. Trindade, G. F., Abel, M. L., Lowe, C., Tshulu, R. & Watts, J. F. A Time-of-Flight Secondary Ion Mass Spectrometry/Multivariate Analysis (ToF-SIMS/MVA) Approach to Identify Phase Segregation in Blends of Incompatible but Extremely Similar Resins. *Anal. Chem.* **90**, 3936–3941 (2018).

4. Keenan, M. R., Trindade, G. F., Pirkl, A. & Newell, C. L. Orbitrap noise structure and method for noise-unbiased multivariate analysis. *Pre-print* (2024).