Supporting Information

Identifying drug metallation sites on peptides using electron transfer dissociation (ETD), collision induced dissociation (CID) and ion mobility-mass spectrometry (IM-MS)

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S-1 Sample preparation

A 2.017 mM solution of the ruthenium anticancer complex, **1,** [($\eta\delta$-bip)Ru(en)Cl]$^+$ in H_2O was prepared. An aliquot of 50 μL of a 493 μM (H_2O) solution of Substance P (Sigma-Aldrich, UK, S-2136) was added to 50 μL of the Ru-drug (final Substance P concentration: 246.5 μM and Ru-drug: 1.085 mM) and incubated for 23 h at 37^{0}C in order to form the Substance P/**1** complex. Following the reaction, the solution was diluted x 100 into 50% aqueous acetonitrile prior to MS analysis.

A 1.0 mM stock solution of cisplatin, **2,** *cis*-[$Pt(NH_3)_2Cl_2$] in H_2O was prepared.
An aliquot of 30 μL of a 493 μM (H_2O) solution of Substance P (Sigma-Aldrich, UK, S-2136) was added to 70 μL of the cisplatin solution (final Substance P concentration: 148 μM and cisplatin: 0.7 mM) and incubated for 23 h at 37^{0}C in order to form the Substance P/**2** complex. Following the reaction, the solution was diluted x 50 into 50% aqueous acetonitrile prior to MS analysis.

S-2 Mass spectrometry

ETD-Ion mobility-mass spectrometry

ESI-MS was performed on a Travelling-Wave based ion mobility-mass spectrometer, the Synapt HDMS (Waters Corporation, Manchester, UK) modified to incorporate ETD functionality. The instrument has been described in detail elsewhere. In brief, mobility section comprises three consecutive, gas filled, travelling wave (T-Wave) RF ion guides. Electrosprayed ions are first accumulated in the first (Trap) T-Wave and then released into the second (Mobility cell) T-Wave, where they undergo mobility separation through action of a continuous train of transient DC voltage pulses (travelling waves). The mobility-separated ions then pass through the third (Transfer) T-Wave into the oa-ToF analyser for mass analysis. Mass spectra were acquired in positive-ion mode. The instrument was operated with a capillary voltage of 3.4kV. The ion mobility T-Wave was operated at 0.5 mbar of nitrogen. The Mobility T-Wave was optimised with a velocity of between 300 m/s and the pulse amplitude ramped between 6-18 V during the acquisition.

Implementation of Electron Transfer Dissociation on the modified IM-MS instrument

Electron Transfer Dissociation experiments were performed on a modified Travelling-Wave research based ion mobility-mass spectrometer, the Synapt HDMS (Waters Corporation, Manchester, UK). In this system, the standard nanoflow electrospray source has been modified to incorporate a fast and efficient intermediate pressure glow discharge reagent anion source. A cross section of the "front end" ETD source block assembly is shown in figure S-1. The isolation valve stem was modified to incorporate a sharpened 1/16th inch stainless steel discharge tube. The additional tube is insulated from the source block, isolation valve and sample cone. A discharge voltage of approximately -300V is applied to the tube relative to the pumping block and a visible glow discharge occurs between the tip of tube and the isolation valve wall. Typically, the discharge current is approximately 35 micro-amps. The tube is connected to a vial containing a few mg of crystalline reagent (not shown). In this study, fluoranthene (*m/z* 202) reagent held at room temperature was used. The tip of the tube is held at an intermediate pressure of 2.2 mbar, consequently, the vial of reagent crystals is held at sub-ambient pressure. In order to regulate the flow of reagent vapour into the discharge region, a make-up gas of

nitrogen flows through the vial and carries the reagent vapour to the tip of the discharge cathode. The make-up gas flow is typically set at 30ml/min. As the vapour passes through the discharge region, copious anions are generated. During reagent ionisation it is necessary to reduce the amount of oxygen and water vapour entering the discharge region. That is achieved by applying a counter flow of dry nitrogen to the front of the sample cone (cone gas).

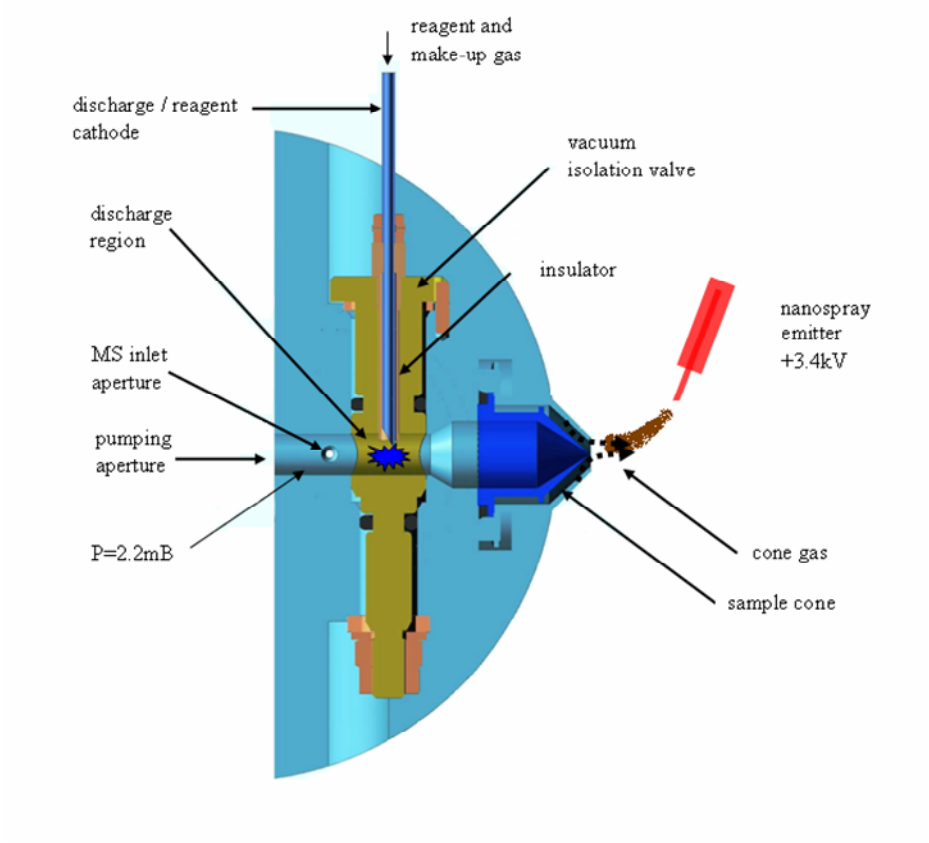

Figure S-1: Schematic of cross-section of source block

During the ETD experiment, cations and anions are sequentially generated. The ion source block temperature was set at 120^{o}C. Solutions of the Substance-P.drug complexes were introduced into the source region of the instrument at the concentration described above in section S-1.
The ion source polarity and the quadrupole set mass were sequentially switched to deliver either triply or quadruply charged precursor cations (as described in main text) and singly charged fluoranthene radical anions (*m/z* 202) into the first (Trap) T-Wave. Analyte cations are generated by applying approximately +3.4kV to the nanospray emitter whilst the reagent cathode is held at the same voltage as the sample cone (typically +30V). Anions are generated by applying approximately -300 V to the reagent cathode relative to the sample cone voltage. Whilst anions are being generated, the nanospray emitter is grounded. Only a few milliseconds are required to switch between either source.

For ETD data acquisition, the TOF analyser accumulated at a rate of 1 spectrum/second and periodically admitted reagent anions into the Trap T-Wave cell for 1 second every 4 seconds. See ion mobility-

section above for details of the instrument. For more efficient ETD, within the Trap T-Wave cell, the bath gas was changed to helium at a pressure of 0.05 mbar. The Transfer T-Wave cell was pressurized to 5e-3 mbar of argon. The T-Wave speed and amplitude influence the ion-ion interaction time as well as the reaction rate. The Trap T-Wave was set at an optimum amplitude of 0.2 V and a speed of 300 m/sec. Studies indicate an optimum reaction time of approximately 20 ms. Data acquisition and processing were carried out using MassLynx (V4.1) in conjunction with custom macro based hardware control software package.

Conventional ion mobility principles and the calculation of collision cross-sections using the T-Wave based ion mobility device

In brief, traditional drift tube ion mobility systems separate ionic species based on differences in the time taken to traverse a gas-filled cell under the influence of a uniform electrostatic field (*E*). The mobility (*K*) of an ion species in a gas is dependent on several factors including the masses of both the ion and the gas, the charge on the ion and the ion-neutral collision cross-section. The average drift velocity (v) that an ion achieves in the gas as a result of the electric field is given by:

$$v = K E \qquad (1)$$

Experimentally K values can be obtained by measuring the time taken for a packet of ions to traverse a drift cell of known length and these values used to determine the CCS (or Ω) values using the theoretically derived relationship:

$$\Omega = ((18\pi)^{0.5}/16)\,(ze/(k_bT)^{0.5})\,(1/m_{ion} + 1/m_{gas})^{0.5}\,1/(NK) \qquad (2)$$

where *ze* corresponds to the charge on the ion; k_b is Boltzmann's constant; *N* is the number density of the neutral gas; the term $(1/m_{ion} + 1/m_{gas})$ is the inverse of the reduced mass relating to the ion and neutral, with m_{ion} and m_{gas} being their respective masses.

The nature of the separation in the T-Wave ion mobility device is more complex than in a standard drift tube mobility experiment since the electric field is neither constant nor uniform. As a consequence, direct determination of mobilities from the measured drift times is not straightforward. Consequently, following the approach taken in other studies, the T-Wave mobility separation has been calibrated using ionic species of known CCS determined using standard drift tubes.

For the T-Wave calibration, the drift tube CCS values were adjusted by multiplying by the square root of the reduced mass and dividing by the charge state of the calibrant species to provide a term related directly to 1/K (see equation 2).

T-Wave ion mobility calibration procedure for the measurement of collision cross-sections

The ion mobility Ω calibration procedure has been described in detail previously by many groups employing T-Wave ion mobility technology[1-10]. The methodology adopted during this study for the measurement of a Ω for the ETD product ions was to calibrate the T-Wave mobility device with ionic species of known Ω determined using standard drift tube instruments.[11] Where Ω values are reported,

the mobility device was calibrated with ions produced a solution polyalanine (1mg/mL in 1:1 H_2O:MeOH) of known Ω.[xi] Details of the calibration procedure were as follows:

Ion mobility Synapt HDMS: experimental for ETD product ion collision cross section measurement

The T-Wave ion mobility drift times were calibrated using singly charged oligomeric ions formed from polyalanine using electrospray ionization (ESI). These ionic species were chosen since their Ωs have previously been measured using standard DC-drift tube mobility instruments[11]. Protonated alanine oligomers, $[Ala_{n= 3, 5-19} + H]^+$ provided a mobility calibration over the Ω range of 89.0-276.3 $Å^2$. The T-Wave ion drift times of the calibrant ions were corrected for their mass-dependent and mass-independent flight times between the T-Wave ion mobility cell and the ToF analyser prior to mobility calibration. The normalised collision cross section (Ω') values [(published Ω * $\mu\sqrt{}$ / z), where μ = reduced mass and z = charge], were plotted against corrected drift time (t'd) values of the calibrant ions. Linear regression gave Ω' = 164.62 t'd + 219.34, r = 0.9998, see Figure S-2.

[Note: The mass-dependent flight time is the time from when the ion exits the transfer T-Wave until it reaches the TOF and is proportional to the $\sqrt{m/z}$ of the ion. The mass-independent flight time is the time taken by an ion to traverse the mobility and transfer T-Wave cells. For a T-Wave velocity of 300 m/s used in this study, this value corresponds to 0.92 ms].

The derived calibration coefficients were then used to calculate the Ωs of the c and $z^{*+\bullet}$ ETD product ions following measurement of their individual drift times (t'd) using the formula:

$\Omega_{T\text{-}Wave} = ((164.62\ t'd) + 219.34)\ z) / \sqrt{\mu}$

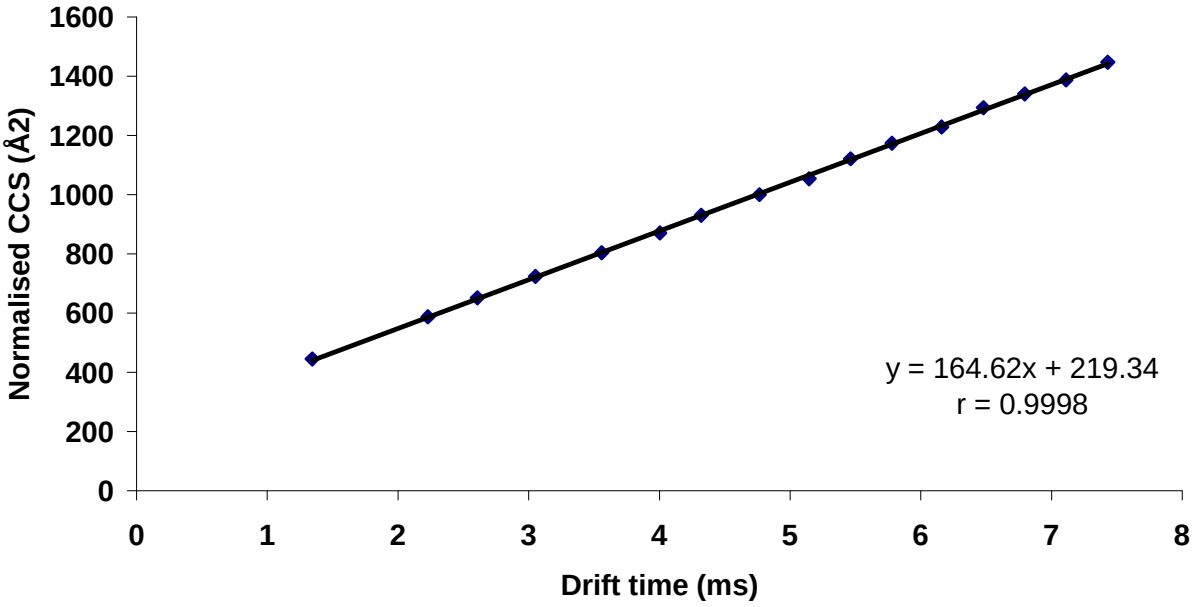

Figure S-2: Plot of the normalised collision cross section (Ω') values against corrected drift time (t'd) values for the oligomers of polyalanine

The polyalanine Ω calibration was validated against singly charged Substance-P, the ruthenium drug (**1**) and the ruthenium drug–HCl ions. The latter two ions were reported[10] recently. Using the coefficients obtained in the calibration procedure, the calculated CCS for singly charged Substance-P was found to be 285 $Å^2$. This compares favorably with the previously reported CCS of 292 $Å^2$ determined using a standard drift-tube technique[11]. The experimentally derived T-Wave CCSs for the ruthenium drug (**1**) and the ruthenium drug –HCl ions were compared to a computational algorithm capable of calculating collisional cross-section values. The algorithm has been explained in detail elsewhere[10] and is based on the Projection Approximation. The algorithm made use of the X-ray crystallographic coordinates for **1** which were further used to form a PDB input file for the generation of a theoretical CCS. The T-Wave measured CCS values for **1** and **1**-HCl were 94 $Å^2$ and 89 $Å^2$, respectively. These measurements are in good agreement with the theoretical CCS calculations of 96 $Å^2$ and 89 $Å^2$ for **1** and **1**-HCl, respectively

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