

1 **Modern Electrospray Ionization Mass Spectrometry**

2 **Techniques for the Characterisation of Supramolecules and**

3 **Coordination Compounds**

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9

10 **Abstract**

11 Mass spectrometry is routinely used for myriad applications in clinical, industrial, and
12 research laboratories worldwide. Developments in the areas of ionisation sources, high-
13 resolution mass analysers, tandem mass spectrometry, and ion mobility have significantly
14 extended the repertoire of mass spectrometrists, however for coordination compounds and
15 supramolecules, mass spectrometry remains underexplored and arguably underappreciated.
16 Here, the reader is guided through different tools of modern electrospray ionization mass
17 spectrometry that are suitable for larger inorganic complexes. All steps, from sample
18 preparation and technical details to data analysis and interpretation are discussed. The main
19 target audience of this tutorial are synthetic chemists as well as technicians/mass
20 spectrometrists with little experience in characterising labile inorganic compounds.

21 **Introduction**

22 Mass spectrometry (MS) has emerged as an integral part of the analytical toolbox and is one
23 of the most routinely used techniques. It has been historically well explored for small organic
24 compounds, as such molecules only contain covalent bonds and are mostly easy to ionise.¹ In
25 the last decades, the focus of MS has expanded rapidly, and two areas have shown particularly
26 big promise: the analysis of complex mixtures, e.g. in “omics”,^{2,3} and the structural
27 characterisation of biomacromolecules in their native state.^{4,5}

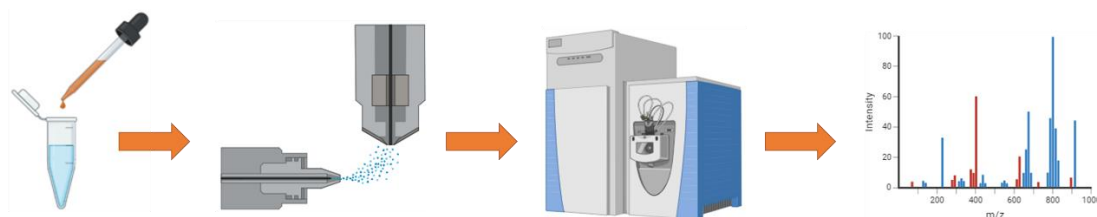
1 Between the extremes of small (organic) molecules and biomacromolecules, the analysis of
2 synthetic inorganic molecules is of particular interest to chemists, including non-covalently
3 bound supramolecules and labile coordination compounds. These systems have become
4 increasingly important in application areas such as medicine, catalysis, materials and in
5 mimicking the functionality of biomolecules.⁶ The structural characterisation of such
6 assemblies is challenging, as X-ray crystallography, NMR spectroscopy or computational
7 methods are often difficult or not feasible for larger structures.⁶

8 Methods adapted from native MS offer a range of advantages for their characterisation over
9 other methods: a) an ultra-high mass resolution, that enables the unambiguous identification
10 of composition and stoichiometry,⁵ b) the robust analysis of complex product mixtures, where
11 different compounds are separated by their m/z , c) the hyphenation with other techniques
12 such as chromatography,^{7,8} ion mobility^{9,10} or spectroscopy^{11,12} and d) the potential to adapt
13 characterisation workflows to an industrial scale if appropriate.⁶ The characterisation of
14 supramolecular and coordination compounds using MS has been reviewed regularly,^{6,13–25}
15 including a book published by Schalley and Springer in 2009,²⁶ however MS is still not
16 commonly and always confidently used in synthetic inorganic laboratories.

17 This tutorial will focus on the practical application of MS (mostly electrospray ionization, ESI)
18 for such inorganic complexes, beginning with technical details as well as potential challenges.
19 The analysis of MS data will further be discussed in detail, before the tutorial ends with an
20 introduction to other gas phase techniques suitable for the investigation of such compounds.
21 While the characterisation of these inorganic systems with MS is less trivial than for small
22 organic compounds, every mass spectrometrists, chemist and technician can learn the
23 necessary skills with the help of this tutorial.

24 **Technical Details**

25 The main aim of the mass spectrometric analysis is to obtain a mass spectrum of the synthetic
26 product. This section will be focussed on technical details to facilitate this process and on
27 pitfalls to avoid (Figure 1).



1.1 Sample Preparation

- suitable solvent
- ionisation reagents
- suitable concentration

1.2 Ionisation Conditions

- (n)ESI, MALDI or CSI
- low temperatures, voltages and flow rates

1.3 Transfer in the Mass Spectrometer

- choice of polarity
- suitable DC and RF voltages, as well as pressures

2. Analysis of the Mass Spectrum

- m/z ratio, charge state
- accurate mass
- isotopic patterns

1

2 **Figure 1:** Mass spectrometric analysis of supramolecules and coordination compounds,
 3 including sample preparation, ionisation conditions, ion transfer and analysis. Abbreviations
 4 are as follows: (n)ESI – (nano)-electrospray ionization; MALDI – matrix-assited laser
 5 desorption ionization; CSI – coldspray ionization; DC – direct current; RF – radio frequency;
 6 m/z – mass to charge ratio. Designed with BioRender.

7

8 Sample Preparation

9 Synthetic products are usually provided as solids or as reaction mixtures in solution, and the
 10 most common ionisation methods start from solution. For solid samples, the choice of solvent
 11 is crucial. As it is challenging for a mass spectrometrists or technician to understand the
 12 intrinsic properties of the product on the same level as the synthetic chemist, ideas for solvent
 13 choices are a valuable input that should be provided. Suitable solvents entirely depend on the
 14 reaction product and its properties – the sample (and potentially the ionisation reagent) need
 15 to be dissolved easily while maintaining suitable concentrations, but without inducing
 16 unwanted disassembly reactions. For labile compounds, it is often advisable to prepare the
 17 solution fresh prior to analysis, which minimises the time for disassembly or other reactions.
 18 It is also important to choose a solvent that is compatible with the ionisation method of
 19 choice. For ESI, this usually involves polar solvents such as water, methanol and acetonitrile,
 20 whereas non-polar solvents are less suitable.²⁷

21 For ESI it is often useful to add salts such as alkali metal halides, or acids/bases, depending on
 22 the product and its lability. This approach can help to enhance the signal of adduct ions $[M +$
 23 $x A]^{x+}$ and $[M + y A]^{y-}$ (with A^+/A^- being e.g. an alkali metal cation or halide anion), or of the
 24 protonated and deprotonated species $[M + x H]^{x+}$ and $[M - y H]^{y-}$, respectively. Buffer
 25 solutions to control the pH value, such as ammonium acetate, which are often used in
 26 biological mass spectrometry, are usually not needed.²⁸

1 The analyte concentration in solution is important, and this is particularly difficult to control
2 when a reaction mixture is the starting point rather than a solid. Modern mass spectrometers
3 are highly sensitive and the optimal sample concentration is decreasing constantly.²⁹ Typical
4 analyte concentrations in modern MS lie in the low μM region.

5 *What are the consequences of using too high concentrations?* One problem is that higher
6 concentrations can cause crystallisation in the solution and at the air-water interface, and
7 when using ESI and nano-electrospray ionisation (nESI), this can often clog the capillary.^{30,31}
8 High concentrations also lead to clustering during the ESI process, and this makes the
9 spectrum more complex and may also not reflect the molecules present in solution.¹⁹ Another
10 potential problem are space-charging effects, which are due to the repulsion of charged ions
11 with the same polarity. This phenomenon can lead to a decrease in accuracy, sensitivity and
12 resolution of the mass^{32,33} (and ion mobility^{34,35}) measurements, although in practice this is
13 usually not a problem for mass. High concentrations lead more likely to oversaturation of the
14 detector,³⁶ which decreases their lifetime, and also means that instruments have to be
15 cleaned more frequently due to the contaminations with neutrals from solution.²³

16 *What are the consequences of using too low concentrations?* Below a certain threshold, the
17 sensitivity of mass spectrometers is not sufficient to detect the analyte. Less obvious is that
18 the formation of coordination compounds and supramolecules is often directed by self-
19 assembly, and this is driven by entropy that in turn depends on concentration.^{23,37,38} Hence,
20 synthetic products can disassemble in solution because the concentration is too low. Most
21 MS efforts are pointless if the analyte is not present in solution, and for some compound
22 classes, the consequences of using high concentrations have to be accepted in order to
23 maintain the analyte in solution. For metallosupramolecular complexes, typical solution
24 concentrations to avoid disassembly are in the high μM and low mM region.^{37,38}

25 It is advised to start at the concentration that is considered ideal for the sensitivity of the
26 instrument. In case the compound relies on self-assembly, and if only smaller fragment peaks
27 are observed but not the analyte signal, the user should gradually increase the concentration.
28 This should lead to the occurrence of larger fragments and eventually the analyte, however
29 aggregates can form at too high concentrations that exceed the mass of the analyte. If no
30 analyte is found, and if no changes occur while increasing the concentration further, the
31 analyte absence can not be explained with solution disassembly.

1 Ionisation

2 The use of soft ionisation methods has enabled the characterisation of large
3 biomacromolecules,³⁹ and the lessons learned from biological MS can be applied to labile
4 inorganic substances.⁶ The most commonly used ionisation source is electrospray ionisation
5 (ESI), where a high voltage is applied to a solution in a capillary. Ions in the liquid migrate to
6 the surface until coulombic repulsion overcomes the surface tension and an ion-solvent cone
7 forms at the tip of the capillary. The detailed mechanism of ionisation depends on the size
8 and structure of the molecule and remains debated,^{40–42} however both the ionisation
9 energy/electron affinity as well as the surface activity play an important role in determining
10 how many ions are formed per analyte species in solution (“response factor”).⁴³

11 A further improvement over ESI for the ionisation of labile complexes to the gas phase is nano-
12 ESI,⁴⁴ for which glass capillaries with a sharp opening in the (sub)micron regime are used.⁴⁵
13 Due to lower flow rates and voltages, and the possibility of lower source temperatures, very
14 large structures such as whole viruses can be ionised, and hence no relevant, upper mass limit
15 exists for synthetic chemists with the exception of large polymers.⁴⁶ The design of these
16 “nano-tips” needs some consideration, and depending on usage, either the purchase of
17 premade nano-tips or the in-house design with capillary pullers is possible. Parameters for
18 common nano-ESI tips can be found elsewhere.^{45,47}

19 For successful ionisation using nESI, a voltage needs to be applied to the solution inside the
20 tip. Two common options exist: the insertion of an inert metal wire (often Pt) that is
21 connected to the source, or the coating of the nano-tip with a conductive material (often Au,
22 Pd or Pt).^{48,49} For most cases, both methods are equivalent. The disadvantage of nESI
23 compared to ESI is the difficulty to hyphenate the technique with liquid chromatography,
24 although this has been partially overcome,⁵⁰ and a tedious tip preparation. Nano-tips are also
25 not perfectly reproducible, which can make the development of robust workflows difficult.⁴⁵

26 Source conditions for labile inorganic complexes need to be soft, which means to avoid in-
27 source fragmentation and to preserve the structure of the analyte. Low flow rates,
28 temperatures and voltages are recommended.^{23,51} There is a trade-off between these soft
29 parameters and those that maximise signal (high flow rates, temperatures and voltages), and
30 finding the ideal parameters may require careful tuning. A key step in the formation of ions

1 *via* ESI is desolvation, which benefits from high pressures and temperatures in the ion source,
2 and also depends on the solvent.⁵² Incomplete desolvation can lead to solvent adducts, which
3 particularly occur with coordinating solvents like acetonitrile.¹⁹ The source temperature is a
4 major factor for preserving the original structure of labile molecules during ESI. Cryo- or cold-
5 spray ionisation sources (CSI) have been developed, achieving good results that seems not
6 easily amenable with ESI.^{53–56} As CSI is not commercially available and only few sources exist,
7 it will not be discussed further.^{57,58}

8 Another ionisation technique is matrix-assisted laser desorption ionisation (MALDI), which is
9 often deployed for synthetic molecules such as organic polymers⁵⁹ and dendrimers.⁶⁰ An
10 important difference to ESI is that fewer multi-charged ions are formed, which facilitates data
11 interpretation. The sample is embedded in an organic matrix, and excited with short laser
12 pulses. After the energy absorption through the matrix and relaxation in the crystal lattice,
13 parts of the sample are desorbed and ionised, and transferred to the mass spectrometer.⁶¹
14 While MALDI has its merit for labile synthetic molecules, it is far less common than ESI. Other
15 ionization methods, such as electron ionisation, chemical ionisation or field ionisation are
16 even less frequently used, but can be suitable e.g. for organometallic compounds.⁶²

17 **Ion Transfer in the Mass Spectrometer**

18 The transfer of ions in the mass spectrometer is realised through a combination of direct
19 current (DC) and alternating radiofrequency (RF) fields.⁶³ The most important consideration
20 is the polarity of the ion optics, as this determines whether cations or anions are transmitted.
21 The preference for either polarity depends on the nature of the sample, its acidity/basicity
22 and ionisation energy/electron affinity.⁶⁴ Biological MS relies overwhelmingly on the positive
23 ion mode, however for synthetic molecules, negative ion mode is also frequently used. MS
24 data collected in negative ion mode are often simpler, less intense and less noisy, due to the
25 availability of fewer ionization pathways.¹⁹

26 There is a trade-off between high ion transmission (using high voltages and low pressures)
27 and preserving the ion structure (using low voltages and high pressures), and the required
28 “softness” of the parameters depends on the rigidity and stability of the sample. The m/z also
29 plays an important role in how well ions are transmitted. Higher m/z ions require higher RF
30 voltages (and lower RF frequencies, but this is usually not user-controlled), but this is not

1 specific for inorganic compounds. More details on tuning mass spectrometers can be found
2 in resources for the specific instruments.⁶³ As modern mass spectrometers are able to
3 transmit ions in the Megadalton regime (1 Dalton = 1 Da = 1 g·mol⁻¹), the size of synthetic
4 compounds should rarely be a limiting factor for ion transfer when properly tuned.⁴⁶

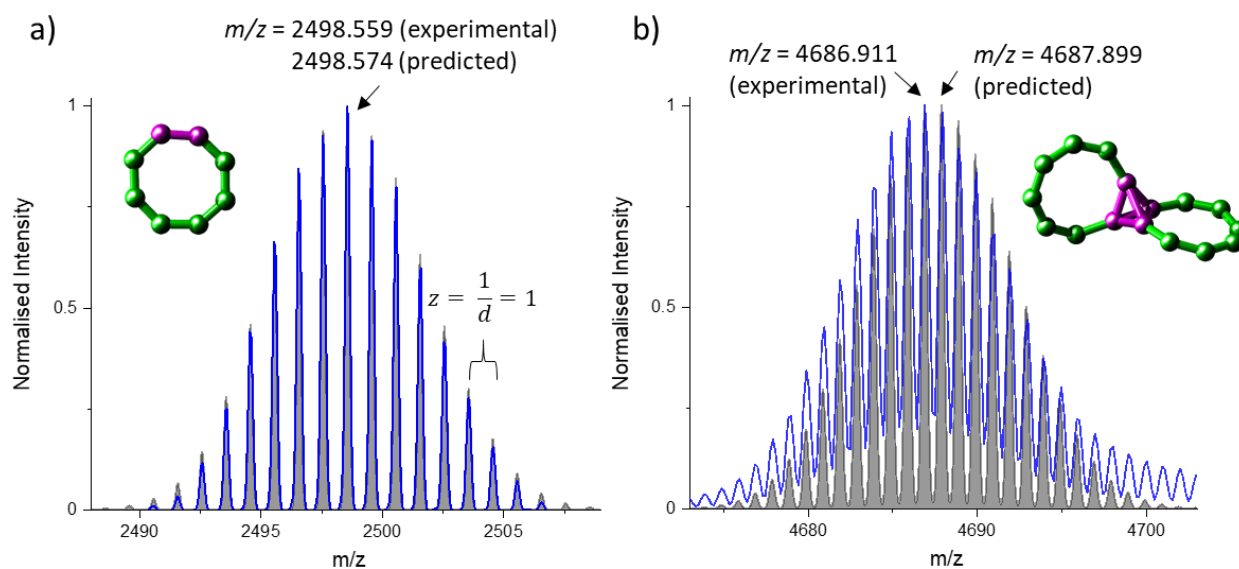
6 Analysis of the Mass Spectrum

7 The measurement of the mass to charge ratio (m/z) is realised in the mass analyser. The most
8 common mass analysers utilised in modern, commercial mass spectrometers are
9 quadrupoles, time-of-flight (TOF) analysers and ion traps.¹ Quadrupoles have the lowest
10 resolution of the ones listed above, and are mainly used for m/z -selection in tandem mass
11 spectrometry experiments or when only a narrow m/z -window is sufficient for analysis. Both
12 TOF and ion traps can have significantly higher resolution than quadrupoles. A well-known
13 example of ion traps is the orbitrap, which delivers high resolution with moderate costs and
14 effort and has hence emerged as a game changer in modern mass spectrometry.⁶⁵ More
15 detailed information about mass analysers can be found elsewhere.¹

16 m/z and Mass

17 High-resolution mass spectra provide wide and rich information that go far beyond the
18 comparison of measured mass with predicted molecular weight. For multiply charged ions,
19 the first step is decoupling the mass m from the charge z . High-resolution mass spectra of
20 most small ions show isotopic distributions, and the distance d between two neighbouring
21 isotopic peaks is related to the charge state of the ion *via* the formula $d = \frac{1}{z}$ (Figure 2a for
22 the example of a {Cr₆Gd₂} ring). Based on the charge state of the ion, the mass can easily be
23 determined by multiplying z with the measured m/z . This approach works usually well, except
24 for when a limitation in resolution or overlapping peaks occur. For the former, the resolution
25 in the mass spectrometer may be increased, e.g. by extending the time the ions spend in the
26 ion trap.⁶⁵ Overlapping peaks can be addressed by deconvoluting the different signals, either
27 manually or with software,⁶⁶ or by using orthogonal separation approaches. A classic example
28 of overlapping species is the combination of the monomer $[M + x A]^{x+}$ and its dimer $[2(M + x$
29 $A)]^{2x+}$, which share the exact same m/z . The only difference between both ion populations is

1 the distance between the isotopic peaks, however the pattern may not be easy to interpret
 2 by eye. Here, a simple deconvolution of the differently charged species is sufficient to
 3 distinguish both ions. More complicated is the separation of isomeric ions of the same charge
 4 state, and ion mobility can be useful for that purpose as it separates ions based on their size
 5 and shape.



6

7 **Figure 2:** Experimental (blue) and predicted (grey) isotopic patterns of a)
 8 $[Cr_6Gd_2F_8(O_2C^tBu)_{16}NH_2^nPr_2]^+$ and b) $[Cr_{12}Gd_4F_{21}(O_2C^tBu)_{28}(NH_2^nPr_2)_2]^+$. Example a) illustrates
 9 how the charge state can be determined by quantifying the m/z distance between two
 10 neighbouring isotopic peaks, leading to $d = z = 1$. The predicted isotopic pattern agrees well
 11 with experiment, and so does the accurate mass of the most intense peak. Case b) is more
 12 difficult, and the unexperienced reader might consider the agreement between experiment
 13 and prediction sufficient. While the digits of the accurate mass are in good agreement with
 14 simulation, the experimental maximum and the whole distribution is shifted to lower m/z . The
 15 agreement is not sufficient to confidently assign this peak to the proposed sum formula. Based
 16 on X-Ray crystallography data, we found that some of the fluoride atoms have likely been
 17 substituted for hydroxyl groups, which suggests an overlap of ions with different numbers of
 18 F atoms and OH groups present.³⁷ Predicted isotopic patterns were simulated with enviPat⁶⁷
 19 based on a ThermoFisher QExactive UHMR at resolution = 12500 (as experiment).

20 Resolution and Accurate Mass

21 Peak overlaps also occur between ions with similar, but not identical m/z , and for that the
 22 resolution of the instrument as well as the accurate mass play an important role. The former
 23 defines how well two given peaks can be separated, and continuous advances in commercial
 24 instrumentation have led to increasing resolving powers.^{68,69}

1 The accurate mass is the experimentally determined mass of an ion with known charge, and
2 the precision of this value depends on the accuracy and precision of the measurement.⁷⁰ For
3 modern orbitrap and TOF mass spectrometers, these ideally agree within 5 ppm (“parts per
4 million”) compared to the predicted mass. Practically, this corresponds to a difference of 1 –
5 3 digits, depending on the size of the ion, the quality of the calibration and the resolution of
6 the instrument (Figure 2a for the example of a {Cr₆Gd₂} ring).

7 It might not be possible for synthetic chemists to control the calibration frequency or quality
8 of an instrument, however the mass accuracy can be determined by introducing a sample of
9 known accurate mass (e.g. NaI or CsI clusters) as an internal standard, which can either give
10 a rough indication of the current mass accuracy or can even serve as the basis for secondary
11 calibration.¹⁹ It can also help to collect a mass spectrum just around the peak of interest,
12 allowing to average as many ions as possible for better accurate mass measurements. The
13 accurate mass can also predict the rough elemental composition of an ion based on the
14 difference to the next full integer (“mass defect”).⁷⁰ For molecules that involve only common
15 organic elements such as H, O, N and C, the accurate mass is usually close to the integer (\pm
16 0.2 Da), whereas transition metals often have larger mass defects and their ions are found in
17 the whole range between two integer m/z units.

18 **Isotopic Distribution**

19 High-resolution mass analysers yield isotopic patterns for small molecules, which occur as
20 most elements involve more than one abundant isotope. When many different atoms are
21 combined, their combination yields a fingerprint-like pattern.¹⁹ Based on the ion’s sum
22 formula and the natural isotopic abundances for a given element, this pattern can be
23 simulated. Comparison of the experimental and simulated isotopic distribution can guide and
24 confirm the peak assignment to a given formula. For small ions including elements with
25 prominent isotopic distributions (e.g. Cl, S, Fe), the pattern can also reveal how many atoms
26 of an element are present. Several online tools are available to predict isotopic distributions,
27 and software from mass spectrometry vendors also often offers this possibility.^{71,72} It is
28 essential to understand that averaged mass spectra, when acquired for a few minutes, can
29 include up to hundreds of millions of ions. With such high ion counts, the statistical foundation
30 of isotopic assignments is very powerful, and an isotopically resolved peak should match the
31 simulated pattern almost perfectly (Figure 2a for the example of a {Cr₆Gd₂} ring). The isotopic

1 pattern also depends on the natural isotope composition of the elements, ion-ion
2 interactions, electronic noise as well as the type of mass analyser and its resolution.⁷³ The last
3 two are taken into account by some online resources, and need to be considered in particular
4 when using high resolution mass-analyzers like the orbitrap.^{67,74,75}

5 A problem occurs when peaks overlap, which results in a deviation from the expected isotopic
6 distribution (assuming they have similar accurate masses of the isotopic peaks, otherwise
7 they lie in between the other ion's signal), as shown in Figure 2b for a {Cr₁₂Gd₄} species. While
8 this can be simulated,⁷⁶ it is also sometimes possible to conclude by eye whether a given peak
9 consists of one or more ions, based on peak shape (e.g. whether there is a "valley" in the
10 isotopic distribution), and differences in the accurate mass.

11 Besides the advantages for peak assignment, the possibility to isotopically resolve peaks also
12 opens a venue for distinguishing a compound from its isotopically labelled species (e.g. with
13 ²H instead of ¹H). This was for example used by Sawada *et al.* to analyse the preference of
14 different host-guest complex enantiomers.⁷⁷

15 **Analysis Approach**

16 Most users assign the mass spectrum manually, although for frequent users software exists
17 that facilitates this process significantly.^{78,76} For any assignment, the *m/z*, accurate mass and
18 isotopic distribution need to agree, and the formula needs to be a reasonable guess
19 containing sensible components, oxidation states, coordination numbers and the correct
20 overall charge. The following procedure is recommended for the analysis of a mass spectrum
21 of the molecule M:

- 22 1. Search for molecular adduct ions⁷⁹ of the formula [M + x A]^{x+} (positive ion mode) or
23 [M + y A]^{y-} (negative ion mode) with one or more charge carrying ions (A = H⁺, Na⁺, K⁺,
24 Cl⁻ etc.). For negative ion mode, anions of the type [M - y H]^{y-} are also often found due
25 to the loss of protons from acidic groups.
- 26 2. Look out for other charge states of the same intact analyte, that could originate from
27 different numbers of charge carriers A⁺/A⁻ or through successive loss of oppositely
28 charged counterions.

- 1 3. Are there other repeating patterns, separated by the same m/z ratio? These can
2 indicate cluster formation or polymeric contaminations. Determine the m/z difference
3 between two neighbouring peaks, and try to assign the pattern. Although these
4 assignments can inform on potential contaminations, it is often more important to
5 avoid them rather than identifying them.
- 6 4. If you are unsure if a given peak originates from the analyte or is a contamination in
7 the solvent, run a mass spectrum of the blank. Several contaminant ions are known
8 for ESI-MS, however, often contaminations are sample-specific.¹⁹
- 9 5. Search for reasonable fragments, e.g. for complexes with loosely bound ligands, as
10 well as ions with varying oxidation states of transition metals. These can occur due to
11 reactions with moisture or through electrochemical reactions during ESI.
- 12 6. If you find ions with higher masses than M , look for oligomers of M or other
13 aggregates.
- 14 7. Try to assign as many of the intense peaks as possible, however, MS is a highly
15 sensitive technique, and it is easy to get lost in the details of complex spectra. Unless
16 there is a specific ion of interest, assign only analyte peaks that are higher than 10%
17 of the most intense peak. Relative intensities can reflect the composition in solution
18 well, although they are influenced by how easy the molecules ionise, their surface
19 activity and by the ions' stabilities in the gas phase.
- 20 8. The peaks that cannot be assigned with steps 1-5 can be subjected to MS^2 (see
21 Tandem Mass Spectrometry). Try to track the fragmentation channels of these peaks
22 down at various collision energies and write down the leaving groups, which will add
23 up to the formula of the original ion.
- 24 9. If it is not possible to identify any peak related to M , it is likely necessary to change
25 solution conditions and ionisation/MS parameters as discussed in the section
26 Technical Details. This could include changing the solvent composition, the charge
27 carrier, the concentration as well as instrumental parameters for ionisation or ion
28 transfer.

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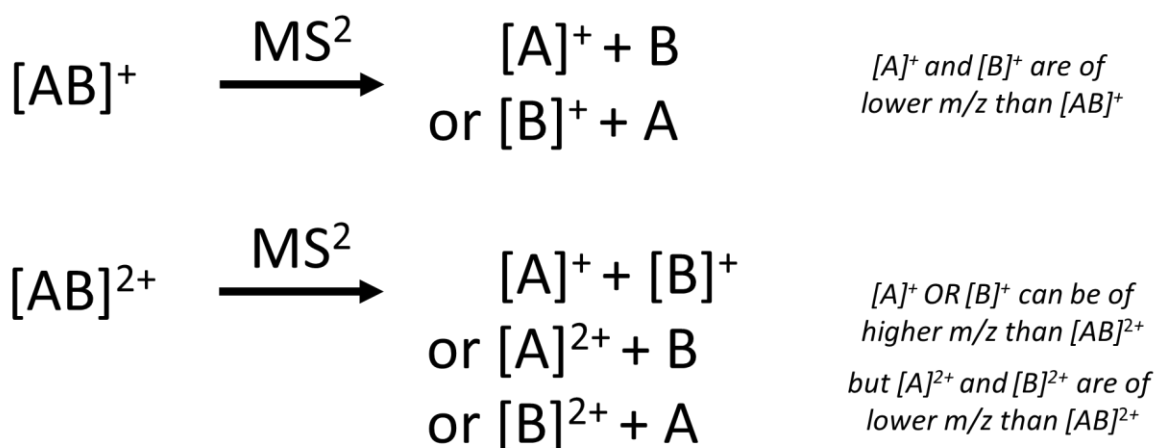
30 **Two-Dimensional Gas Phase Separation Approaches**

31 **Tandem Mass Spectrometry**

1 Most commercial mass spectrometers can perform tandem mass spectrometry (MS^2)
2 experiments. In an MS^2 experiment, ion populations are selected based on their m/z
3 (commonly with a quadrupole), and subsequently activated, which usually results in smaller
4 fragment ions that are in turn mass-analysed. The activation of ions can be realised *via*
5 energetic collisions with inert gas (collision-induced dissociation, CID) or surfaces (surface-
6 induced dissociation); electron- (electron capture or transfer dissociation) or photon-
7 mediated (ultraviolet-photodissociation or infrared multiphoton dissociation), as well as with
8 other methods.^{80,81} The most common MS^2 technique is CID, in which ions are accelerated
9 into a collision cell filled with a stationary inert gas (e.g. N_2 , Ar, Xe). After every gas collision,
10 translational energy of the ion is converted to vibrational energy, which is distributed
11 throughout the ion, usually leading to the dissociation of the weakest bonds. Other tandem
12 mass spectrometry activation methods are not as widespread or not even commercially
13 available, and are beyond the scope of this article.⁸⁰

14 The main information from CID experiments is the composition of fragment ions and
15 indirectly of the leaving groups (fragment mass = precursor mass – leaving group mass), and
16 the stoichiometries of the fragments can inform on the structural subunits present in the
17 original precursor ion and their connectivities. Fragments can further dissociate to secondary
18 fragments, and it is important to select collision energies appropriately.

19 The fragmentation of singly charged species occurs from high to low m/z , involving the loss
20 of neutral leaving groups that lead to singly charged fragment ions. An ion of the structure
21 $[AB]^+$ can fragment to $[A]^+$ and the neutral B, or to $[B]^+$ and A. Both ions $[A]^+$ and $[B]^+$ are at
22 lower m/z than the precursor $[AB]^+$ (Figure 3). As neutral leaving groups cannot be detected,
23 data visible in the mass spectrum is biased towards structures that retain charge, which is
24 influenced by their size and structure. For the fragmentation of multiply charged species, the
25 charge state z can change, leading to fragment ions at higher m/z (but lower z) than the
26 precursor. It is possible that both products of the precursor retain a charge.¹⁹ An ion $[AB]^{2+}$
27 can dissociate to $[A]^+$ and $[B]^+$, and due to the reduction of the charge state z , either of these
28 ions (but not both simultaneously) can be at higher m/z than the precursor (Figure 3).



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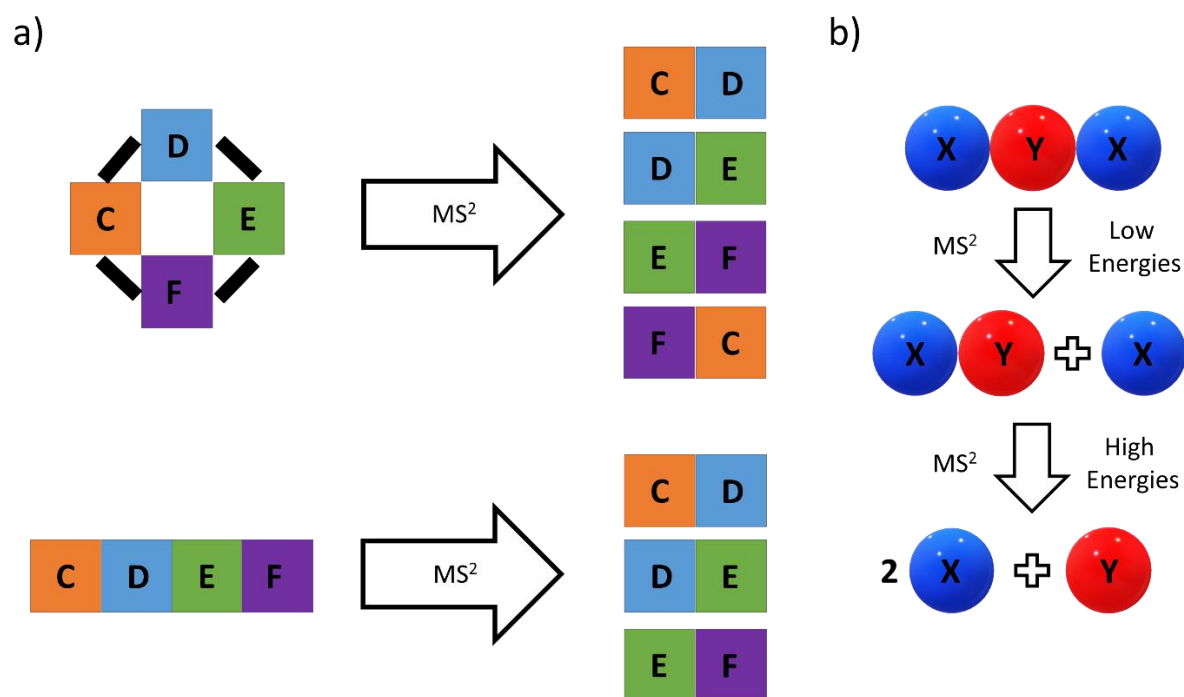
2 **Figure 3:** Fragmentation of the singly charged $[AB]^+$ and the doubly charged $[AB]^{2+}$. For $[AB]^+$,
 3 the fragment ions $[A]^+$ and $[B]^+$ are found at lower m/z than the precursor. The dissociation of
 4 $[AB]^{2+}$ can result in $[A]^+$ or $[B]^+$ at higher m/z than the precursor, due to the loss of charge,
 5 whereas $[A]^{2+}$ and $[B]^{2+}$ are always at lower m/z than $[AB]^{2+}$.

6 Two hypothetical cases are discussed, in which the analytes consist of the subunits C, D, E, F,
 7 and X, Y, respectively (Figure 4). These examples are based on the assumption that all subunits
 8 cannot dissociate further, and that the ions are equally likely to retain the charge upon
 9 fragmentation.

10 The precursor ion consists of C, D, E and F, and the ions CD, DE, EF, and FG are found in the
 11 MS^2 spectrum (Figure 4a top). From this data, it can be derived that C is likely linked to D and
 12 F; D is linked to C and E; E is linked to D and F; and F is linked to E and C. The most sensible
 13 explanation is a circular structure of the type CDEF, in which C and F are connected as well,
 14 whereas the absence of the ion CF would have indicated a linear connectivity (Figure 4a
 15 bottom). Real examples are more complex, and structural rearrangements can occur and
 16 need to be taken into account when deriving structural information from MS^2 spectra.^{82,83} In
 17 practical examples, the units C, D, E and F might also not be static, and distinguishing the
 18 linear from the cyclic species might be possible by measuring differences in the mass of the
 19 potentially terminal units C and F.

20 Another factor are the relative intensities of fragments, which can inform on two points: 1)
 21 *Likely fragments statistically occur more often.* In a chain XYX, the fragments X, Y and XY could
 22 be observed (Figure 4b). Fragment Y will likely have the least intense signal at low collision
 23 energies, as both X-Y bonds have to be broken. Conversely, both X and XY will occur with

1 similar intensities after the first fragmentation step, as every broken X-Y bond results in equal
 2 amounts of X and XY. 2) *Stable fragments are more abundant.* XY is potentially less stable than
 3 X as it can dissociate a second time to X and Y, whereas X cannot further fragment. Hence,
 4 the relative intensities of the fragments would follow the order $X > XY > Y$, until at high
 5 fragmentation energies XY disappears completely and twice as many X are present relative to
 6 Y (Figure 4b).



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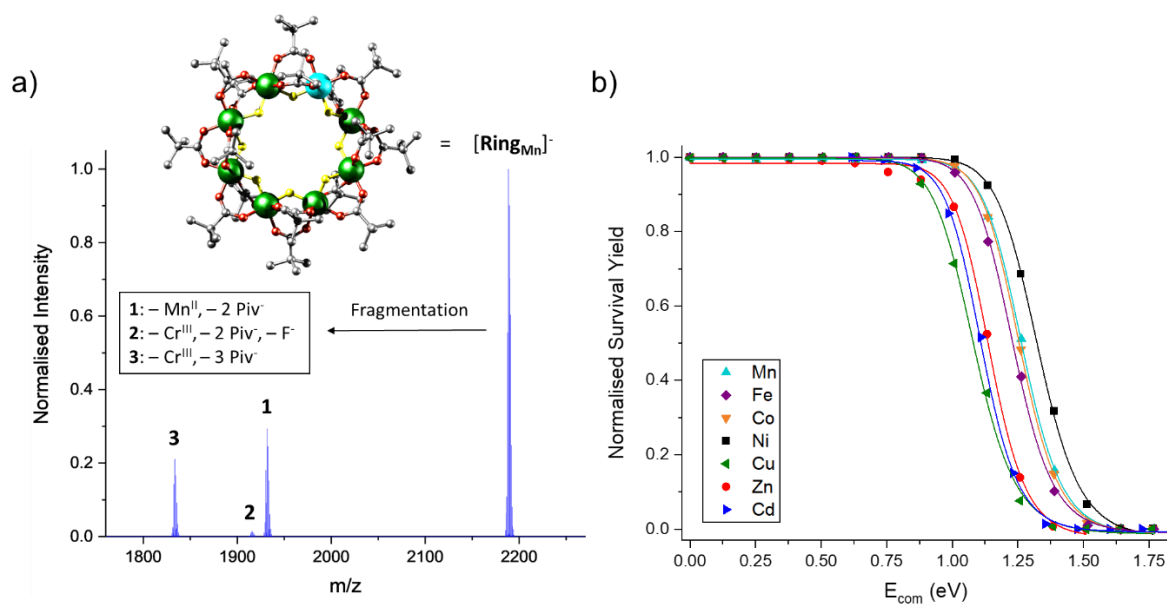
8 **Figure 4:** a) Fragmentation of CDEF with either a cyclic or linear structure. The presence or
 9 absence of CF can inform on the connectivity of CDEF. b) Fragmentation of the chain XYX to
 10 the fragments $XY + X$ at lower and $2X + Y$ at higher collision energies, respectively. The relative
 11 abundances of X, Y and XY can inform on the precursor structure.

12 The appearance of fragment ions is dependent on the collision voltage, which multiplied by z
 13 corresponds to the collision energy E_{lab} . This energy is user-defined and stable ions require
 14 higher collision energies to fragment, and varying the energy can investigate ion structure
 15 and stability. A good illustrating example is a macrocycle, which can coordinate guests at two
 16 different locations: in the inside (“endo”) leading to a host-guest complex, or on the outside
 17 (“exo”). Kiesilä *et al.* investigated the structure of a pyridine[4]arene dimer Z_2 that
 18 simultaneously coordinates a hexafluorophosphate anion (PF_6^-) and an acetone molecule.⁸⁴
 19 Using MS^2 , the authors found that almost exclusively PF_6^- is lost in the first fragmentation step
 20 at low collision energies, suggesting that PF_6^- is less strongly bound and hence *exo*-

1 coordinated (Figure S1 right). In turn, this indicates that acetone is encapsulated into **Z₂**
2 (“endo”), which was later confirmed by X-Ray crystallography (Figure S2). Taken together, this
3 example illustrates how MS² experiments can distinguish different isomers based on their
4 stabilities.

5 Stabilities can also be quantified using MS² by ramping the collision energy and plotting it
6 against the “survival yield” (SY), which represents the share of precursor ions that does not
7 fragment. These curves are usually S-shaped, and certain points in the fitted graphs can be
8 determined, e.g. the *E*₅₀ value where 50% of the precursor fragment. These values can be
9 regarded as relative measures of ion stability.^{85,86} While they have a thermodynamic meaning
10 for some non-commercial instruments (“guided ion-beam mass spectrometers”),⁸⁷ for
11 commercially available platforms these depend on the instrument, pressures, voltages and
12 ion structure. The latter is important as large structures experience more collisions and the
13 mass determines the energy that gets transferred during the collisions. These effects can
14 interfere with thermodynamic properties, which is why *E*₅₀ and similar values should hence
15 be regarded as semi-quantitative data, which are best used for the interpretation of trends
16 between similar ions.

17 One example is the fragmentation and stability trend of the polymetallic rings
18 [Cr₇M^{II}F₈(O₂C^tBu)₁₆]⁻ = [**Ring_M**]⁻ (Figure 5a Inset), in which seven Cr^{III} and one divalent metal
19 (M^{II} = Mn^{II}, Fe^{II}, Co^{II}, Ni^{II}, Cu^{II}, Zn^{II} and Cd^{II}) are bridged *via* fluoride and pivalate ligands (O₂C^tBu⁻
20 , Piv⁻). The dissociation of these anions proceeds through multiple channels, and for [**Ring_{Mn}**]⁻
21 this involves the loss of Mn^{II} and two Piv⁻ (to **1**), or the loss of Cr^{III}, one F⁻ and two Piv⁻ (to **2**) or
22 three Piv⁻ (to **3**) (Figure 5a). The isostructural ions with other M^{II} fragment similarly, and as
23 kinetic affects are likely small, differences in the stability curves (Figure 5b) and *E*₅₀ values are
24 associated with thermodynamic trends. *E*₅₀ differences of up to 22% were found between the
25 most stable M^{II} = Ni^{II} and the least stable Cu^{II}, which were rationalised with trends from crystal
26 field theory.⁸⁸



1

2 **Figure 5:** (a) MS² data of [Ring_{Mn}]⁻ at E_{lab} = 110 eV. Inset: structure of [Ring_{Mn}]⁻ (Cr: green,
 3 Mn: cyan, F: yellow, O: red, C: gray). Hydrogen atoms in the tert-butyl groups were omitted
 4 for clarity. (b) Normalized survival yield vs E_{com} for [Ring_M]⁻ fitted to a sigmoidal function (M =
 5 Mn: cyan, Fe: purple, Co: orange, Ni: black, Cu: green, Zn: red, Cd: blue). E_{com} is the collision
 6 energy in the center-of-mass frame and is a more precise representation of the energy that is
 7 transferred during single ion-gas collisions. Reproduced from ref. 88, © 2022 The Authors.

8

9 Investigating the stability of compounds is not easily achievable with other techniques, and
 10 the unique feature of MS² is that solvent molecules and counter ions do not interfere, making
 11 it possible to decouple these effects from the actual analyte stability.^{6,25} MSⁿ experiments
 12 (with n > 2) are possible, although not always available in commercial instruments (mostly in
 13 instruments with certain types of ion traps). This technique can investigate the disassembly
 14 and stability of already fragmented ions.⁸⁹

15 Ion Mobility Spectrometry

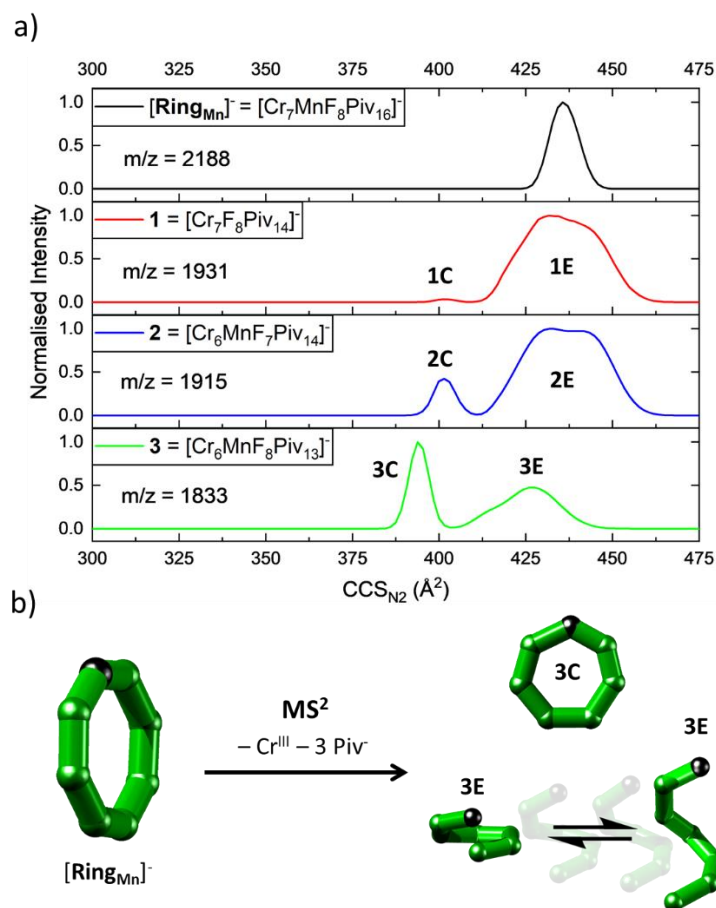
16 Ion mobility (IM) is increasingly used in commercial instrumentation, separating ions based
 17 on their size, shape and charge. In the easiest form, ions move through a gas-filled drift cell
 18 guided by an electric field. The electric field is usually not strong enough to induce
 19 fragmentation, however, collisions with the buffer gas still occur, the more the larger the ion
 20 is. The collisions determine the time the ion spends in the drift cell, and by injecting ion pulses
 21 and measuring this time, structural information is gained. The drift (or arrival) time can be
 22 converted to a collision cross section (CCS) value, which is comparable across instruments and

1 to values computationally simulated from candidate geometries (e.g. based on density
2 functional theory or molecular dynamics calculations).^{9,25} The community has witnessed a
3 significant increase in the use of IM, however it is still not available or used in the majority of
4 MS laboratories. In particular synthetic chemists and facility technicians often do not have
5 access to such platforms, and this technique will hence not be discussed in detail.^{9,10,25,90} The
6 interested reader is referred to a recent perspective on using IM-MS for synthetic
7 molecules.²⁵

8 IM can be an important tool to distinguish isomers, for example binding sites in
9 supramolecular complexes. One illustrating example is the ternary complex with **Z₂**, PF₆⁻ and
10 acetone, that was already discussed above.⁸⁴ MS² showed that the *exo*-coordinated PF₆⁻
11 dissociates more easily from **Z₂** than acetone, which suggested that PF₆⁻ is *exo*- and acetone
12 is *endo*-coordinated (Figure S1 right). IM can directly give the same information, and
13 measuring the *CCS* values of several ions involving **Z₂** showed that those including PF₆⁻ are
14 larger than those that do not involve PF₆⁻ (Figure S1 left). As an *exo*-coordinated species will
15 impact the size of **Z₂** more than an encapsulated one, this strongly supports that PF₆⁻ is *exo*-
16 coordinated, in contrast to acetone.

17 The study of the polymetallic rings [**Ring_M**]⁻ (Figure 5) further illustrates the insights that IM
18 can offer when combined with MS². The size and shape of fragments **1-3** (Figure 6a) were
19 measured, and for all three species, two *CCS* distributions were found: one at lower *CCS* and
20 narrow (**C**, “compact”) and one with a wider peak shape at higher *CCS* (**E**, “extended”). Based
21 on computational structure optimisations and *CCS* simulations, these were assigned to closed
22 rings (**C**) and conformationally dynamic, open structures (**E**, Figure 6b).⁸⁴ Taken together,
23 these two examples highlight the richness of structural data that can be obtained from IM,
24 and it is anticipated that its application in synthetic laboratories and facilities will significantly
25 increase in the future.²⁵

26 [**Ring_M**]⁻ can encapsulate ammonium cations to form rotaxanes, and the case example of the
27 polymetallic rotaxane **Am_{Mn}** will be discussed in the Supporting Information, including sample
28 preparation, MS data analysis, as well as MS² and IM measurements and interpretation.



1

2 **Figure 6:** a) CCS_{N_2} Distributions of $[Ring_{Mn}]^-$ and fragments (1–3) at $E_{lab} = 110$ eV. b)
 3 Fragmentation of $[Ring_{Mn}]^-$ to **3** including structural assignments of **3C** to closed heptametallic
 4 rings and **3E** to conformationally dynamic, open structures. Reproduced from ref. 88, © 2022
 5 The Authors.

6 4. Conclusions

7 The combination of soft ionisation sources (mainly ESI), high-resolution mass analysers and
 8 commercially available MS^2 and IM additions has enhanced our understanding of
 9 coordination compounds and supramolecules. MS is an active field of analytical research, and
 10 while the discussion here is limited to techniques that are established and commercially
 11 available, other methods such as ion spectroscopy^{11,12,91} or ion soft-landing including
 12 microscopic imaging^{92,93} yield further, unique structural information. In my view, all these
 13 techniques will gain in importance for supramolecules and coordination compounds in the
 14 future. Overall, this article should be regarded as a guideline on how to make the most of the
 15 MS toolbox for labile inorganic compounds, and I hope this tutorial inspires synthetic chemists
 16 and technicians to analyse such molecules more frequently and with confidence.

1 **Conflict of Interests**

2 There are no conflicts to declare.

3 **Supporting Information**

4 MS data for the example of the ternary complex involving Z_2 ; Case example for the
5 characterisation of the heterometallic rotaxane Am_{Mn} using different MS methods

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16 **Biography**

17 Dr. Niklas Geue is a postdoctoral researcher in the groups of Prof. Perdita Barran and Prof.
18 Richard Winpenny at The University of Manchester, UK. He received his Bachelor degree in
19 Chemistry from Leipzig University in Germany in 2019, before he worked on different projects
20 in Analytical Supramolecular Chemistry at the Pontificia Universidad Católica de Chile, the
21 University of New South Wales in Sydney and the University of California, Los Angeles. He
22 obtained his PhD as a President's Doctoral Scholar from The University of Manchester in 2023,
23 where his project focussed on the use of ion mobility mass spectrometry and tandem mass
24 spectrometry for the characterisation of metallosupramolecular complexes. His postdoctoral
25 work now involves the development of ion soft-landing and microscopic imaging for
26 biomacromolecules and supramolecules. Niklas also serves as a committee member of the
27 British Mass Spectrometry Society (BMSS), a board member of the eLeMeNte e.V. society and
28 as an advisory board member of the Friends of the Chemistry Olympiad in Germany (FChO).

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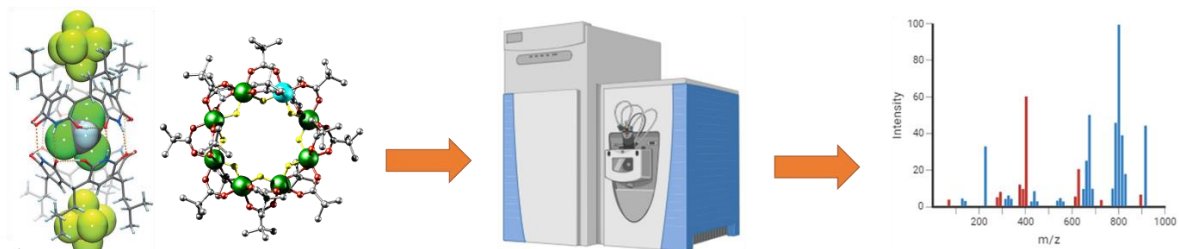
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