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Software requirements for the analysis and interpretation of native ion mobility mass spectrometry data

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Abstract

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5 The last few years have seen a dramatic increase in applications of native mass and ion mobility spectrometry, especially
6
7 for the study of proteins and protein complexes. This increase has been catalysed by the availability of commercial
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9 instrumentation capable of carrying out such analyses. As in most fields, however, the software to process the data
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11 generated from new instrumentation lags behind. Recently, a number of research groups have started addressing this by
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13 developing software, but further improvements are still required in order to realise the full potential of the datasets
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15 generated. In this perspective, we describe practical aspects as well as challenges in processing native mass spectrometry
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17 (MS) and ion mobility-MS datasets, and provide a brief overview of currently available tools. We then set out our vision
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19 of future developments that would bring the community together and lead to the development of a common platform to
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21 expedite future computational developments, provide standardised processing approaches and serve as a location for
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23 the deposition of data for this emerging field. This perspective has been written by members of the European
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25 Cooperation in Science and Technology Action on Native MS and Related Methods for Structural Biology (EU COST
26
27 Action BM1403) as an introduction to the software tools available in this area. It is intended to serve as an overview for
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29 newcomers and to stimulate discussions in the community on further developments in this field, rather than being an in-
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31 depth review. Our complementary perspective (also in this issue) focuses on computational approaches used in this
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33 field.
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Introduction

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5 A thorough understanding of biological processes at the molecular level requires a detailed study of proteins, their
6 complexes, and their interactions with other classes of molecules such as DNA, lipids, carbohydrates and other small
7 molecules. A large number of diseases arise from proteins being unable to carry out their physiological functions ¹.
8
9 Since the development of soft ionisation techniques, in particular electrospray ionisation ², mass spectrometry (MS) has
10 rapidly been adapted for the study of proteins, protein-ligand and protein-protein complexes. Further advances in sample
11 preparation and instrumental developments – such as the use of modified quadrupoles, lower voltage potentials, source
12 temperatures, higher vacuum pressures and volatile aqueous solvents such as ammonium acetate – resulted in the field
13 now referred to as native MS ^{3,4}.
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24 In native MS, the analyte of interest (e.g. protein, nucleic acid, noncovalent complex) is prepared in an electrospray-
25 compatible buffer, and is transferred to the gas phase in the form of ions, with instrument settings selected so that the
26 perturbation of the solution structures is minimized ^{3,5,6}. An increasing number of studies confirm that, under carefully
27 controlled conditions, the native-like structure of proteins and their complexes can be preserved ⁷⁻⁹, while some structural
28 collapse has been observed for some biomolecules ^{10,11}, a specific example being antibodies ¹². Native MS experiments
29 provide information regarding the conformation, composition, stoichiometry and interactions of proteins. The analyses
30 are very fast and sensitive, and they make it possible to simultaneously characterise several species present in complex
31 samples, even from mixtures isolated directly from complex biological matrices ¹³⁻¹⁵.
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43 Incorporation of ion mobility (IM) separation into native MS experiments further enhances the information obtained on
44 proteins and protein complexes. IM separates ions based on their interaction with a buffer gas as they travel through a
45 collision cell under the influence of a weak electric field. The time an ion takes to traverse the IM cell is related to the
46 charge and rotationally averaged collision cross-section (CCS or Ω) of the ion, the latter being a physical quantity related
47 to the overall shape of the ion ^{16,17}. The IM-MS combination allows one to separate coexisting forms of a given
48 protein/protein complex, even within the same charge state, that would otherwise be indistinguishable using either MS
49 or IM alone ¹⁸. This combination has also allowed in depth studies on the conformation of protein ions following the
50 electrospray process and has provided evidence that the solution structure is preserved into the gas phase ^{7,9}.
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While the field of native IM-MS is still relatively new, the last few years have seen a rapid expansion in the number of studies using this methodology, partially fuelled by the introduction of commercial instrumentation (**Figure 1**).

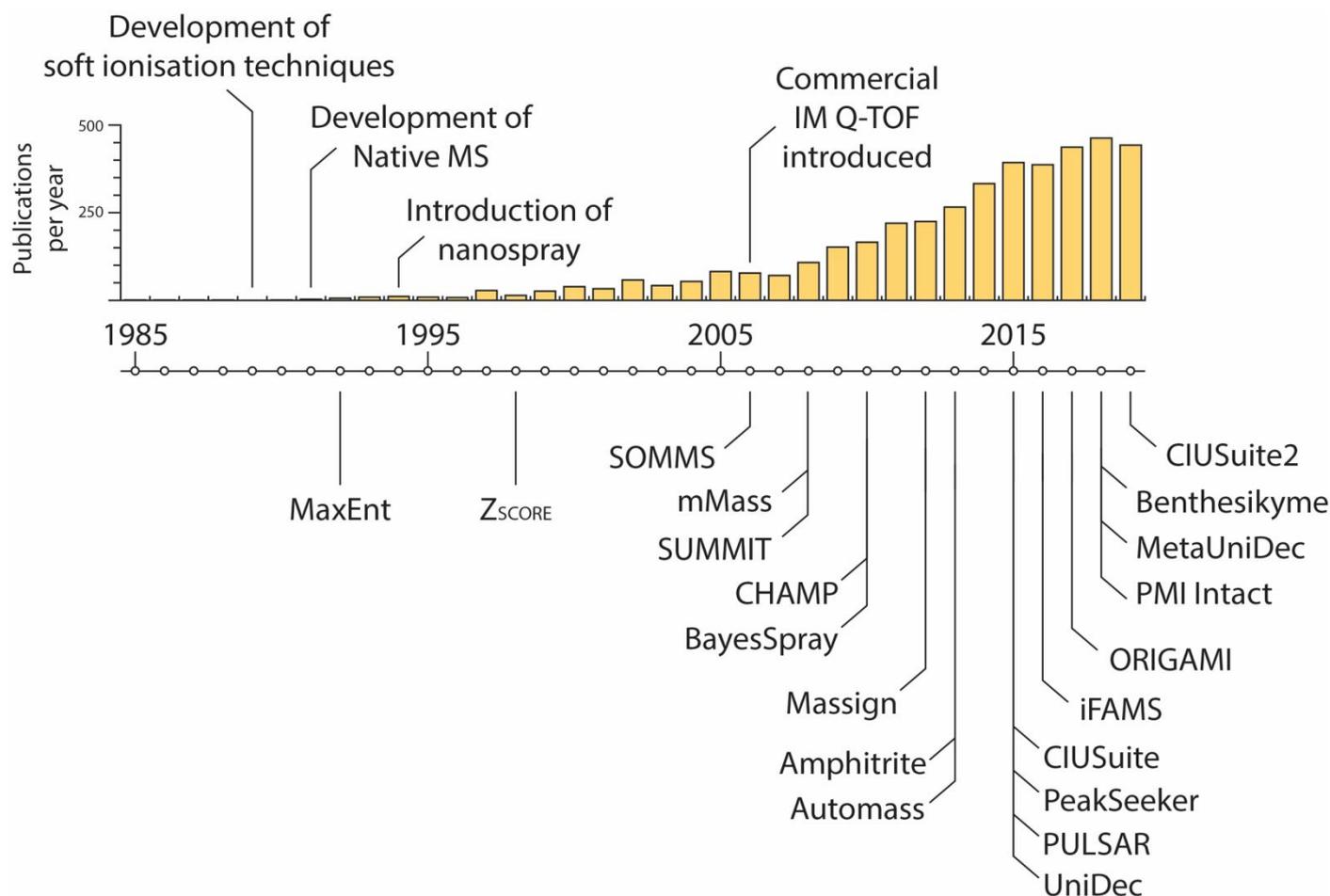
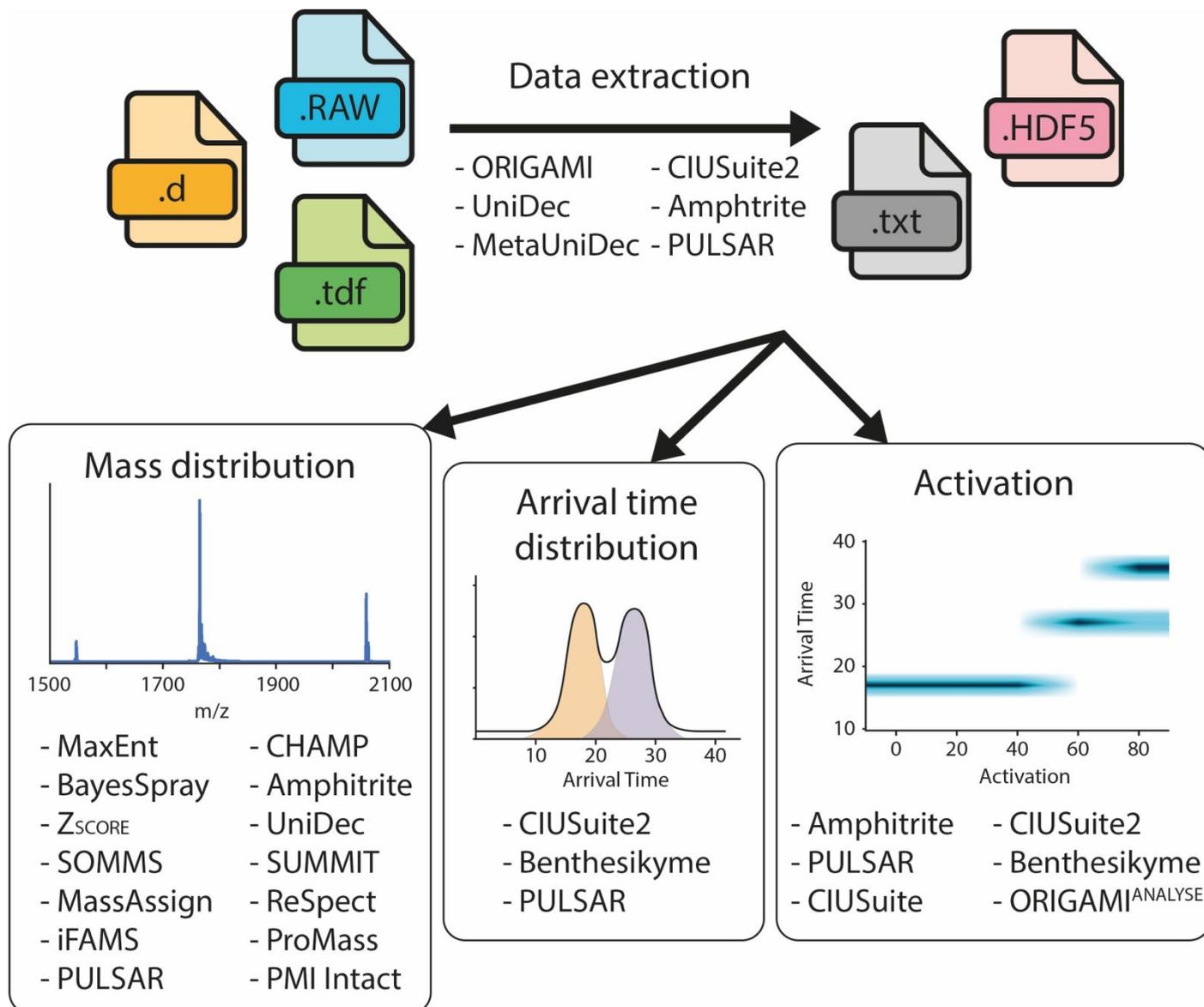


Figure 1: Timeline of major developments in native MS (top) and associated supporting algorithms and software (bottom). Yearly publication metrics were generated from Web of Science in March 2020 by searching for topic: (“ion mobility” AND “mass spectrometry”). Software tools and their features are listed in Table 1.

Though the software offered by vendors is particularly useful for thorough processing of MS data, in particular that involving denatured proteins, it provides only basic functionality and still requires further manual processing routines (which are often slow, cumbersome and error-prone), to process native MS data (which by nature are broader, exhibit fewer charge states, and can contain overlapping distributions from other proteins). MS software developed for the areas of proteomics, lipidomics, glycomics and metabolomics is more mature in nature, however such tools are not optimised for the types of analyses required for native IM-MS datasets. Specialised software providing automated processing approaches, apart from yielding considerable time savings, would increase the reproducibility of processing and analysis. It would also facilitate the exchange of results between laboratories and provide robust documentation of how experiments were carried out and how the data were processed.

1 Although in recent years significant progress has been made in the development of specialised native MS and IM-MS
2 software ¹⁹⁻²⁸ (**Figure 1 – Table 1**), further improvements are required to make full use of the data and cement the role
3 of native MS and IM-MS as standard tools in structural biology investigations. Here we describe the challenges in
4 processing native MS and IM-MS data, and provide an overview of existing software and their features. We then
5 highlight current shortcomings and outline possible solutions along with our vision for the future of this field. This
6 perspective article has been written by members of the European Cooperation in Science and Technology Action on
7 Native Mass Spectrometry and Related Methods for Structural Biology (EU COST Action BM1403), a group focused
8 on developing and applying new biomolecular MS methods to make the characterisation of protein structure and
9 dynamics more rapid and routine. It is intended as an introduction to the software tools available for native mass and
10 ion mobility spectrometry for newcomers to this field. Discussions we have had with members of the community
11 indicated that such an introduction would be a useful addition to the literature, particularly for life scientists now
12 developing an interest in using this technology. We have written a separate perspective article (also published in this
13 issue), which serves as a companion paper to this one and focuses on introducing computational approaches used in the
14 field. In writing this perspective, we also intend to encourage the community to nucleate around a set of shared goals in
15 order to capitalise on our collective expertise and enable more efficient innovation in this growing and very important
16 area.

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36 ----- Box 1 -----
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38 **Box Figure 1:** From raw data to usable formats. Vendor data is converted to text or other file formats for downstream
39 analyses. The types of analyses performed and the corresponding software offering such functionality are shown.

41 MS-based methods yield raw spectral data stored in instrument manufacturers' proprietary formats. The supporting
42 software programs supplied by these manufacturers can usually perform actions such as basic viewing and signal
43 processing (e.g. smoothing, background subtraction, peak picking). Integration of vendor-processed spectral data into
44 custom workflows and researcher software requires manual data export, making this route cumbersome and non-viable
45 for automated and high-throughput analyses.

47 Software developed by research groups typically incorporates methods to read raw data stored in proprietary file formats
48 (**Table 1**). Many of the early studies utilising native MS and IM-MS were conducted on Waters instruments, as they
49 were the first to introduce a commercial IM instrument to the market²⁹. For this reason, early software such as
50 *Amphritrite*, *PULSAR*, and *CIUSuite2* have custom-built file readers that accept the raw files as input and can read them
51 by calling the Waters dynamic linked library (cdt.dll) (**Box Figure 1**). *CIUSuite2* more recently has also added
52 functionality for reading Agilent raw files. Alternatively, programs such as *ORIGAMI^{ANALYSE}* utilise data extraction tools
53 available within the Waters ion mobility software Driftscope. Due to the development of high-mass Orbitraps (made by
54 Thermo Scientific) and the utility of such instruments for native MS applications, software such as *UniDec*, *MetaUniDec*
55 and *ORIGAMI^{ANALYSE}* also accept Thermo Raw files. Alternatively, other software such as *Benthesisikyme* and *iFAMS* use
56 as input text files that have been exported by using the manufacturer's software.

57 While there are many open data formats available in the MS community, these have not been adopted by the native MS
58 field. This is because the design of current open data formats has been chosen for more common applications of MS in
59 ACS Paragon Plus Environment

various proteomic, lipidomic, and metabolomic fields. Depending on the type of analysis, be it MS or MS/MS, eXtensible Markup Language (XML)-based formats such as *mzData*, *mzML*, *mzXML* are commonly used and interchanged. However, these tend to better cater to MS and not IM-MS metadata. In instances where IM is supported, the syntax tends to be too verbose in the full profile mode. Another open data format alternative is Unified Ion Mobility Files (UIMF)³⁰, which is based on a relational database management system. However, UIMF was mainly developed for IM proteomics applications and, thus, does not allow storing experimental data types such as those from activation experiments. So, overall none of the formats described above is ideal for storing data from native IM-MS experiments, which is why none of them has been broadly adopted as a standard in the community.

Recently, the open data format Hierarchical Data Format (HDF5) has been used by *MetaUniDec*. The ability to group together multiple types of data objects into a single file, offered by HDF5, appears to offer the right flexibility to store the types of native MS and IM-MS experiments described here. The HDF5 format also allows one to store many of the experimental parameters, crucial for reproducibility and transparency. As part of the same EU COST Action that is behind this article (BM1403), the community has recently put forward recommendations about which such parameters should be included¹⁷.

----- Box 1 -----

The dimensionality of native IM-MS data

To understand the challenges in processing native IM-MS data, we first describe the different dimensions of the data (see **Figure 2**). By this, we refer to dimensions by which the ions are separated and we include ion intensity as an implicit dimension. We then describe what processing is required, available software for such processing, and the biological information gained (for overview see **Box Figure 1**). While native MS analyses can be interfaced with prior chromatographic separation^{31,32}, this is not routine yet and so we do not include chromatographic retention time as a dimension.

1st dimension – mass to charge ratio (m/z)

The first dimension of separation is the mass-to-charge (m/z) ratio. Such information is obtained from native MS experiments of a protein sample even when heterogeneous or polydisperse. As the primary ionisation method for such analyses is electrospray, and in particular nano-electrospray, what is observed for each protein is a population of charges, referred to as a charge state distribution (CSD)³³. Native mass spectra can therefore be complex, especially when multiple proteins are present in the sample, each resulting in their own CSD which can often overlap. Further complications arise as charge states are also typically not isotopically resolved, which impairs charge state assignment for each peak. CSDs are linked not only to the mass but also to the structural compactness of the detected ions³⁴⁻³⁶.

2nd dimension – ion mobility separation

Incorporation of IM adds an extra dimension of separation, as each m/z species is separated according to its transit time through an ion mobility cell. What is recorded by the instrument is the arrival time, which is the time an ion spends in

the mobility cell plus the time to travel from the exit of this cell to the instrument detector. The transit time in the mobility cell is related to the mobility of the ion, itself linked to its CCS.

3rd dimension – ion reactivity

A third dimension can be added by perturbing the structure of native-like ions by increasing their internal energy. The most frequently used activation methods include collision-induced dissociation (CID)^{37,38}, surface-induced dissociation (SID)^{39,40} or ultraviolet photodissociation (UVPD)⁴¹⁻⁴³. Other methods include electron capture dissociation (ECD) and electron transfer dissociation (ETD)⁴⁴. In these cases, IM can monitor structural rearrangement by recording the change in the observed arrival time distribution (ATD) as a function of ion internal energy, or input energy.

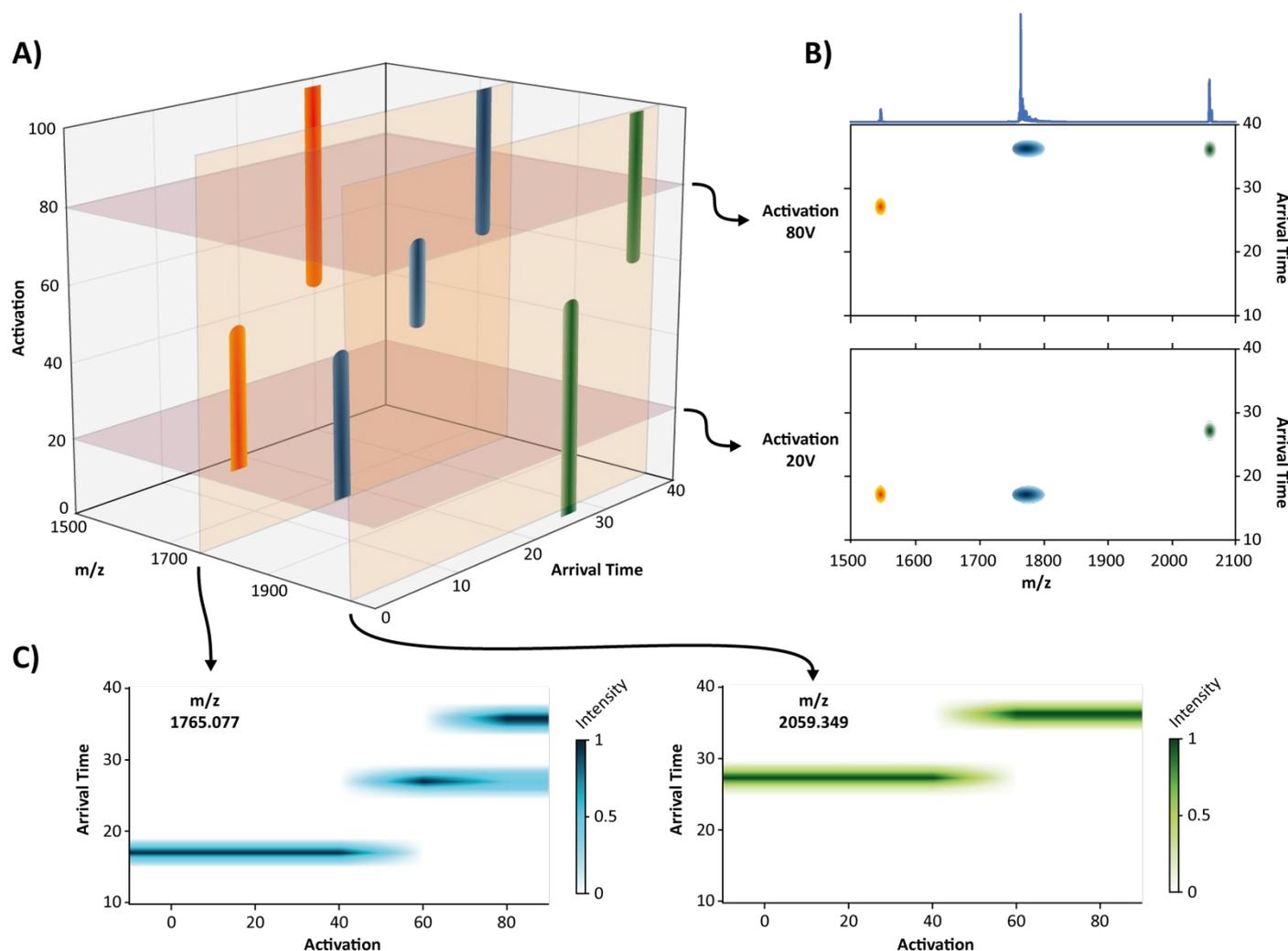


Figure 2: A) Figure illustrating the different dimensions present in a native IM-MS experiment namely, m/z , ion mobility separation and ion reactivity. 2D plots of binary representations of this 3D dataset are shown in B) and C). B) shows the arrival time corresponding to each species in the native MS spectrum, at different activation voltages (20 and 80V). C) Evolution of arrival time distributions for selected m/z species due to activation conditions.

Processing approaches

1 As with mass spectra from other types of analyses, some standard processing of native mass spectra, such as smoothing
2 and background subtraction, is required. This is done after the raw data, almost always recorded in proprietary data
3 formats, are extracted into a more usable format (see **Box 1**). Once the data have been extracted and made available in
4 an accessible format, the following processing steps can take place.
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10 Peak assignment – from m/z to mass distribution

12 A simple way to calculate the mass from a CSD is to select the peak tops of what are presumed adjacent charge states
13 of a given species and solve two equations to find mass and charge (see **Supporting Information Figure S1**). This
14 process, however, becomes difficult when multiple overlapping peak series are present in the spectrum. Initially, semi-
15 automated deconvolution algorithms were created for processing electrospray spectra – *MaxEnt* and later others such as
16 *ReSpect* and *Z_{SCORE}* – with these algorithms being incorporated in commercial vendor software. Freely available
17 standalone tools, such as *SOMMS*, *Massign*, and *CHAMP*, were later developed by research labs to start addressing the
18 issues posed by native MS data. More recently, both academic-developed programs such as *Amphitrite*, and *UniDec*,
19 and commercially-developed ones such as *ProMass*, *PMI Intact* have improved on previous methodologies. Typically,
20 they simplify and speed up the deconvolution process by automatically picking, fitting and assigning charge states to
21 protein and protein-complex samples. The peaks for each charge state, which typically lack isotopic resolution, are
22 usually described by Gaussian distributions. However, other distributions, such as Lorentzian or mixed distributions,
23 have also been used as the experimental peaks can have a pronounced tail due to adducts attachment. Most software
24 simulate a spectrum and then use least squares optimisation to minimise the differences between the simulated and
25 experimental data. *UniDec* uses a Bayesian deconvolution algorithm. A Bayesian approach has also been recently used
26 to improve upon the original *MaxEnt* algorithm, with the new algorithm now referred to as *BayesSpray*. *iFAMS* uses a
27 Fourier Transform-based algorithm for spectral deconvolution.
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49 Native MS experiments can provide the mass of a protein or protein complex, and are the gold standard in reporting on
50 the oligomeric or ligand-binding state of a protein. Knowing the mass of a protein complex can also provide insights
51 into its possible composition, especially if the masses of the individual components comprising the complex are known.
52 Software such as *SUMMIT*⁴⁵ has been developed to do this. Measurements of mass changes due to small molecule or
53 ligand binding can also be used to determine the stoichiometry of such binding events.
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1 As well as measuring composition and stoichiometry, native MS can also be used to determine apparent binding
2 affinities⁴⁶. For example, one can determine the affinity of a ligand binding to a protein if one assumes that intensities
3 reflect concentrations in solution of the bound and unbound species. These affinities are calculated by fitting the relative
4 ion intensities of bound and unbound forms as a function of the ligand concentration. Accurate extraction of the
5 ion intensities, or peak areas, of different bound forms is therefore required^{47,48}. Deconvolution of the data allows one to
6 use the extracted peak areas for this purpose, which can provide more accurate data for fitting than the peak tops.
7 Software such as *UniDec* provide multiple models to fit data and enable the calculation of both micro- and macroscopic
8 K_d values.

19 Extraction of arrival time distributions

21 Extraction of the arrival time distribution (ATD) for a particular ion (i.e. the ATD associated with a specified m/z range),
22 is facilitated by the deconvolution of the mass spectrum (described above). From the deconvoluted spectrum, the m/z
23 range corresponding to each peak is known, which can then be used to extract the corresponding range in the arrival
24 time space (i.e. the ATD), and the charge z will be used to convert arrival times to CCSs. Once an ATD is extracted, its
25 apex can be obtained, which is usually the main descriptor of an ATD. Further peak fitting, typically using one or
26 multiple Gaussians, can also take place. This is useful as the width of an ATD reports on the conformational
27 heterogeneity of an ion. In other cases, deconvolution of the ATDs can reveal the relative distribution of overlapping
28 conformational families²⁰. *CIUSuite2* performs this deconvolution in a semi-automated manner while programs like
29 *Benthesikyme* do this using a genetic algorithm. Most programs fit the minimum number of Gaussians required explain
30 the experimental data without taking into account the theoretical diffusion limited peak-width. This is an active area
31 of research and future implementations should take this into account.

32 Monitoring changes in ATD features, e.g. the widths or relative populations of deconvoluted conformational families,
33 can reveal insights into the effects of mutations or ligand binding events on protein structure²⁰. Most importantly,
34 combined information about folding and binding can be retrieved for each resolved component of the ATD profile.
35 There are cases, however, where the ATD alone does not reveal significant changes. In these instances, activating
36 particular charge states and monitoring changes in the ATDs as a function of activation energy provides further
37 information³⁸.

60 Activation

1 Activation experiments usually involve stepwise increases in collisional energies. This elicits conformational changes
2 in the protein ions, resulting in changes in the recorded arrival times in the IM domain ⁴⁹. For protein-ligand complexes,
3 the ligand can dissociate either prior to or, more commonly, after protein unfolding. Such experiments can report on the
4 strength of this protein-ligand interaction, and how far ligand binding depends on the preservation of local structure of
5 a binding pocket in the protein. These changes are typically visualised by the generation of ‘unfolding plots’, which
6 stack the extracted ATDs for a particular ion as a function of the applied activation energy. Several programs have been
7 developed to generate these unfolding plots (*Amphitrite*, *PULSAR*, *CIUSuite*, *Benthesikyme*, *ORIGAMI^{ANALYSE}*). The
8 ATD for each voltage is usually normalised to the highest intensity and smoothed prior to being used to generate the
9 two-dimensional plots. Even though CID is the most commonly used method in these experiments, the methods
10 previously mentioned in the 3rd dimension – ion reactivity section can also be used.

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13 *CIUSuite* and *CIUSuite2* have functions to identify features of unfolding trajectories that describe or ‘fingerprint’ a
14 given protein’s characteristics. Many software (*CIUSuite*, *CIUSuite2*, *PULSAR*, *ORIGAMI^{ANALYSE}*) can also generate
15 difference and root-mean-square deviation plots to allow unfolding profiles to be compared. *Benthesikyme* provides
16 summary plots that capture the changes in average arrival time and in the full width at half maximum of the ATD during
17 activation experiments and uses these to compare samples.

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23 The voltages at which transitions occur between discrete species in unfolding plots can be affected by the bound state
24 of an ion. For example, ligand binding can induce conformational stability in the various observed forms, analogous to
25 in-solution thermal shift unfolding/aggregation experiments. *PULSAR* implements an equilibrium unfolding model,
26 similar to those used for solution studies. Fitting of unfolding plots to this model can be used to determine the activation
27 levels where transitions are observed between discrete species in an unfolding plot. This information can be used to
28 identify the mid-transition voltage between different conformational and/or association states of a protein. *CIUSuite2*
29 identifies features from replicate experiments and models the transition between these features using a sigmoid function
30 to identify the mid-transition voltage. Unfolding curves can be used to report on the effects of mutations and ligand
31 binding (e.g. small molecules, metal ions, peptides, lipids) on protein stability, and to compare the structural properties
32 of highly similar proteins.

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59 Converting arrival time distributions to collision cross sections
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1 IM measures the ATDs of individual ions, and these values can be used to obtain CCSs, either via first principles or via
2 calibration approaches ¹⁷. This step requires first the extraction of the ATD for a particular ion, *i.e.* the ATD associated
3 with a specified *m/z* range. Prior deconvolution of the mass spectrum as described above aids with this step. ATDs can
4 be further processed to smooth the data and to fit peaks (e.g. by Gaussians), in order to locate the centroids of the arrival
5 time peaks. For experiments involving calibration, the peak centroids for a set of calibrant ions are identified and used
6 to construct a calibration curve ¹⁷. Some programs have the CCS values of selected calibrant ions pre-loaded, while
7 others allow one to use calibrant values of choice. *Amphitrite*, *PULSAR* or *ORIGAMI^{ANALYSE}* automate many of the tasks
8 required for calibration. The goal of the calibration is to provide an equation relating the arrival time to the CCS. Possible
9 improvements in the future would include reporting on the uncertainties associated with the calibrated values, in line
10 with recently published recommendations ¹⁷. Once a calibration has been performed, the ATD axis can be converted to
11 a CCS axis.

22 Harnessing IM-MS data for structural biology

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Once an experimental CCS has been obtained, it can be compared to the CCSs for molecular structures obtained by
other experimental methods, such as X-ray crystallography or NMR, or models provided by computational methods.
The development of methodologies and related software to estimate the CCS of a molecular structure constitutes a
theoretical and technical challenge on its own, and is an area of active research. An in-depth discussion of these
methodologies, software implementing them and how to parameterise this software is beyond the scope of this
perspective article. The reader is referred to a recent review on this topic ¹⁶. Briefly, CCS calculation software originally
focused on calculating the CCS from atomistic models given prior information about the radius, and sometimes partial
charge, of each atom. More recently, however, efforts to calculate the CCS electron density maps represented as point
clouds ^{50, 51} or super-coarse-grained models ⁵²⁻⁵⁷ have been proposed. These methods are especially useful when no
atomic information about the proteins of interest is available (**see companion perspective (REF)**).

Comparison of experimental and theoretical CCS values has been used extensively to report on the gas-phase structure
of ions and whether the ions are able ^{7, 9}, or not ⁵⁸, to retain their solution-phase structure. Recent work has focused on
including CCSs in integrative modelling pipelines ^{56, 59-62}.

Many of the data from native MS and IM-MS experiments can be highly complementary to data obtained by other
structural biology methods, and can be exploited in molecular modelling scenarios. CCSs can be used to validate
structures produced by, for example, homology modelling, coarse-grained, or molecular dynamics simulations ⁶³. MS

1 and IM-MS data can also be fed directly into integrative modelling software programs to guide the refinement of the
2 most likely conformation(s) of quaternary assemblies ⁶⁴. Data typically exploited in this context include (1) detailed
3 stoichiometric information about the subunits and complex partners and (2) CCS distributions. Stoichiometric
4 information is used to define how many subunits should be manipulated during a protein docking run. Information about
5 subunit connectivity within a complex, calculated with programs such as *CHAMP*, as well as CCS data, are also used
6 as restraints in modelling protocols ⁶⁴⁻⁶⁶.
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15 **Perspective and future directions**

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17 The recent growth in software for native MS has provided various tools, which have independently implemented many
18 similar functionalities. There is now an opportunity for us as a community to consolidate best practice in the approaches
19 and methods used for the preparation and processing of data. This is a necessary step to ensure the integrity and
20 reproducibility of native IM-MS results across different instrument platforms and laboratories, as well as to define future
21 data reporting standards. This will require establishing a set of benchmark data files that can be used to evaluate the
22 capabilities of the available software and how they compare with respect to the biophysical quantities they report on.
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32 First, a common data format will need to be agreed upon (see Box 1). With the advent of commercial IM-MS
33 instruments, the need to move towards an open MS/IM-MS format is apparent. The interface between vendor proprietary
34 software and post-processing software developed in the research community is not straightforward in all cases.
35 Manufacturers need to concentrate support on the most common applications of their technology, but this can have the
36 unfortunate consequence of impeding access to the raw data needed by those in the research community who develop
37 their own specialised analytical tools. A common file format, into which vendor formatted data can be easily extracted,
38 should be accompanied by common tools for reading and writing in that format, analogous to what the *msconvert* tool
39 provides for the proteomics community ⁶⁷.
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51 It is also important that the file format records meta-data (i.e. experimental parameters, including sample preparation,
52 and relevant instrumental parameters). These are important for the correct reproduction of experiments, and also for
53 adherence to policies regarding the storage and management of research data ⁶⁸. A proposal outlining relevant
54 information that needs to be captured in IM has recently been put forward ¹⁷.
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1 A common file format would enable the creation of a data repository and encourage the deposition and meta-analysis
2 of data. This would have significant benefits for computational researchers as well as experimentalists, and further
3 support data sharing and transparency in data collection. Establishing a repository for native IM-MS data would provide
4 opportunities to link these data with entries on other structural repositories such as the PDB ⁶⁹, EMDatabank ⁷⁰, PDB-
5 Dev ^{71,72}, and pE-DB ⁷³, as well as on proteomics databases such as PRIDE ⁷⁴ and Uniprot ⁷⁵. We would also welcome
6 the inclusion of native IM-MS outputs such as mass, ATD and CCS, under well-defined experimental conditions, among
7 the parameters describing protein structures in these databases.
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17 While these initiatives would consolidate capabilities that current software provide, developments in MS hardware
18 continue to occur at a rapid pace, as along with the coupling of upfront separation methods to native MS workflows ⁷⁶,
19 ⁷⁷. Examples are the development of higher resolution in MS and IM dimensions, tandem ion mobility analysers, and
20 multistage activation and separation experiments ^{78, 79 80 81 82}. To accommodate these and any future innovations, a
21 common programming library is needed that accomplishes common processing tasks such as reading raw files,
22 smoothing, background subtraction, Gaussian envelope fitting, visualisation and data export. This would reduce
23 duplications in software development, speed up development time and enable more focus on innovation in data analysis.
24 This library would preferably be written in the Python programming language, due to its flexibility, accessibility, and
25 existing codebase. It should be made available on code-hosting platforms such as GitHub or Bitbucket to allow for more
26 sustainable software, better code testing, and greater collaboration between research groups. Using common tools for
27 analysis should also improve the reproducibility of results. Having such a common codebase in a publicly accessible
28 repository would also allow the community to further develop and extend the software together, and would allow any
29 developments to be made available immediately to the community.
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47 Once the software library and common file format are in place, a further development would be to create a software tool
48 for accessing/visualising these files, similar to PRIDE Inspector, which is used to access proteomics datasets ^{83,84}. Recent
49 improvements in web technologies mean it is now possible to create a platform-agnostic, web-based front end for the
50 visualisation of native IM-MS datasets. This front-end would be web-accessible via a browser and mobile devices, and
51 could provide a barebones environment implementing common processing routines. A plug-in interface could allow the
52 integration of algorithms for data processing. This front end could integrate access to CCS calculation software and
53 other software that is currently needed for interpreting native MS and IM-MS data, such as new gas-phase molecular
54 dynamics approaches. The latter is an underdeveloped area, but very important for fully exploiting the data; we
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1 anticipate a lot of algorithm and software innovations to take place in this field in the coming years. Currently, tools for
2 calculating the theoretical CCS of molecular structures are used as standalone programs. Integration of these calculators
3 with software for the processing of raw data would allow a more straightforward comparison between experimental
4 observations and theoretical models, and would allow the parameters for both approaches to be stored in one common
5 format, again assisting in transparency and data reproducibility.
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12 We have set out ambitious aims with regards to data standardisation, the development of software tools and the creation
13 of a common platform for the IM-MS field. We feel these steps are important for facilitating data sharing and
14 collaboration, and for encouraging future innovation, transparency and reproducibility in this area.
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21 Practically speaking, examples of how these aims could be achieved are the Collaborative Computational Projects
22 (funded by the UK's Engineering and Physical Sciences Research Council) that exist in other structural biology fields,
23 e.g. X-ray crystallography, NMR spectroscopy, electron microscopy. A similar initiative in IM-MS could also engage
24 the broader structural mass spectrometry community – including those involved in cross-linking, covalent labelling or
25 Hydrogen-Deuterium eXchange, as these techniques provide complementary information about protein structures and
26 do not yet have a standardised format or dedicated data repository. A community-driven software framework will
27 establish a solid platform for integrating native IM-MS data into the wider structural biology and biophysics
28 communities.
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40 **Associated content**

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42 Supporting Information is available: Figure S1, Example of simultaneous equations to solve the charge state and, from
43 that, calculate the mass of a given species in the spectrum.
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Table 1: List of the software available to process Native MS and/or IM-MS data.

Name	Website	Interface	MS	IM-MS	Activated IM-MS	Accepted file formats	Language	Licence	Release year	Reference
MaxEnt	Contact Vendor	GUI	x			.raw (Waters)	N/A	Proprietary	1992	85
ProMass	Link	GUI	x			.raw (Waters); .dwiff (Sciex) ; .raw (Thermo)	N/A	Proprietary	2001	
Xtract & ReSpect	Contact Vendor	GUI	x			.raw (Thermo)	N/A	Proprietary	n/a	
SOMMS	N/A	Command-line	x			.txt, Text	Perl	Free academic	2006	86
mMass	Link	GUI	x			Text, msD	Python/C	Free academic	2008	87
SUMMIT	N/A	N/A	x			N/A	N/A	N/A	2008	45
CHAMP	Request from authors	Command-line	x			.txt, Text	Python	Free	2010	27
BayesSpray	Contact Vendor	GUI	x			.raw (Waters)	N/A	Proprietary	2010	88
Massign	Link	GUI	x			.txt, Text	LabView	Free academic	2012	28
Amphitrite	Link	GUI	x	x	x	.raw (Waters); .csv, Text	Python/C	Free GNU General Public Licence	2013	26
Automass	N/A	Command-line	x			N/A	N/A	N/A	2013	25
CIUSuite	Link	Command-line		x		.txt, Text	Python	Free academic	2015	23
PeakSeeker	Link	Command-line	x			.txt, Text	Python	Free	2015	89
PULSAR	Link	GUI		x		.raw (Waters)	Python/C	Free	2015	24
UniDec	Link	GUI; Command line	x			.raw (Waters); .txt, Text	Python/C	Free academic	2015	22
iFAMS	Link	Command line	x			.txt, Text	Python	Free academic	2016	90
ORIGAMI	Link	GUI	x	x	x	.raw (Waters); .raw (Thermo); .csv, Text	Python/JavaScript	Free academic	2017	21
Benthesikyme	Link	GUI; Command line		x	x	.txt, Text	JavaScript/Python/C /CUDA	Free	2018	20
MetaUniDec	Link	GUI; Command line	x		x	.txt, Text	Python/C	Free academic	2018	91

1											
2	PMI Intact	Link	GUI	x			all vendors	C++	Proprietary	2018	92
3											
4	CIUSuite 2	Link	GUI; Command line	x	x	x	.raw (Waters); .txt, Text; .d (Agilent)	Python	Free academic	2019	19
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