Evolution of Orbitrap Mass Spectrometry Instrumentation

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Annu. Rev. Anal. Chem. 2015. 8:61–80

The *Annual Review of Analytical Chemistry* is online at anchem.annualreviews.org

This article's doi:
10.1146/annurev-anchem-071114-040325

Keywords

high-resolution, accurate mass, mass analyzer, MS/MS, data-independent analysis

Abstract

We discuss the evolution of Orbitrap™ mass spectrometry (MS) from its birth in the late 1990s to its current role as one of the most prominent techniques for MS. The Orbitrap mass analyzer is the first high-performance mass analyzer that employs trapping of ions in electrostatic fields. Tight integration with the ion injection process enables the high-resolution, mass accuracy, and sensitivity that have become essential for addressing analytical needs in numerous areas of research, as well as in routine analysis. We examine three major families of instruments (related to the LTQ Orbitrap, Q Exactive, and Orbitrap Fusion mass spectrometers) in the context of their historical development over the past ten eventful years. We discuss as well future trends and perspectives of Orbitrap MS. We illustrate the compelling potential of Orbitrap-based mass spectrometers as (ultra) high-resolution platforms, not only for high-end proteomic applications, but also for routine targeted analysis.

INTRODUCTION

MS: mass spectrometry

FT-ICR: Fourier transform ion cyclotron resonance

Innovation in mass spectrometry (MS) is driven by both the scientists that specialize in the development of mass spectrometers and the scientists who use mass spectrometers as tools. Since the initial release of the first Orbitrap-based mass spectrometer in 2005, the expanding needs of the MS community have continuously inspired, challenged, and contributed to the further development of Orbitrap-based MS technologies.

This review discusses the birth and evolution of Orbitrap instruments and how continued development of the novel mass analyzer has been influenced and driven by the changing world of modern analysis. The current prominence of the Orbitrap analyzer is a result of the interplay of several factors including the development history, its characteristic combination of analytical qualities, and the evolution of MS-based applications.

The variety of Orbitrap-based instruments that have been developed reflects the breadth of applications for which the Orbitrap analyzer has been utilized. These applications span from routine screening and quantitation analyses to complex analytical challenges, ranging from proteomics to structural ID and characterization. This review, which coincides with the tenth anniversary of the commercialization of Orbitrap-based instruments, attempts to place the development of various Orbitrap technologies into appropriate historical and analytical context.[1]

FROM IDEA TO REALITY: PROOF OF PRINCIPLE FOR THE NOVEL ORBITRAP MASS ANALYZER

As with many novel mass spectrometer technologies, the Orbitrap mass analyzer has a long and convoluted development history, stemming back to the principle of orbital trapping introduced in 1923 (1). Kingdon described a trapping device consisting of a charged wire stretched along the axis of an enclosed metal can. The charged wire establishes an electrostatic field within the can, and ions that possess sufficiently high tangential velocity orbit the wire, rather than directly colliding with it. As Perry et al. (2) review, subsequent work confirmed that charged particles could be trapped in these electrostatic fields; however, exploiting this technique for mass analysis had not yet been considered or attempted.

Further progress in the field of electrostatic trapping included improvements in ion optics for electrostatic fields (3, 4) and, as Knight (5) explains, successful orbital trapping of laser-produced ions. The Knight experiment included crude mass analysis performed by applying axial resonant excitation to trapped ions and ejection to a detector near the axis, outside of the trap. Unfortunately, significant improvements to this device were required to resolve even simple mixtures.

The development of the first Orbitrap mass analyzer was driven by the desire to build a novel spectrometer that would avoid the shortcomings of prior instruments, such as the complexity and size of Fourier transform ion cyclotron resonance (FT-ICR) analyzers; the low sensitivity, dynamic range and (at the time) resolution of orthogonal time-of-flight (TOF) analyzers; and the limited mass accuracy of ion trap analyzers.

On the basis of this motivation and the substantial body of work on electrostatic traps, Makarov (6, 7) set out to improve the definition of the trapping field, perform ion injection from an external ion source, and create a detection scheme that was compatible with the structure of the trap. The first orbital trapping mass analyzer was designed by replacing the thin wire, previously used as the central electrode, with a spindle-shaped central electrode in combination with two symmetrical

[1]A valuable source of information on Orbitrap MS-related applications and literature is the online library at the Planet Orbitrap website (**http://www.planetorbitrap.com**).

outer electrodes that precisely complemented the shape of the central electrode. The outer electrodes, which were electrically isolated from each other, were used for two purposes: establishment of the ion trapping field and receiver plates for image current detection. The inner and outer electrodes were machined with the highest accuracy possible to maintain fine control of ion motion. The geometry of the trap was optimized to maximize the sensitivity of the image current detection and reduce higher harmonics. To enable injection of externally generated ions (originally utilizing a pulsed laser to produce ions), an elaborate slot was machined with a compensation electrode (a deflector) that minimized ion losses during transfer. With ions successfully entering the orbital trap and oscillating around the central electrode, the image current from coherently oscillating ions was detected on the receiver plates as a time-domain signal. A mass spectrum was generated by Fourier transform of this signal and applying a simple two-point calibration. This newly developed device, later named the Orbitrap™ mass analyzer, joined the Fourier transform family of mass analyzers, then consisting of a Fourier transform TOF MS (8), Fourier transform ion traps (9), and the highly regarded FT-ICR mass spectrometer (10). The first two types of Fourier transform mass spectrometers existed only as home-built, relatively low-performance setups; therefore, it was natural to expect that the latest newcomer would also spend decades in a similar infancy stage.

Although the first prototype instrument incorporating an Orbitrap mass analyzer indeed represented a successful proof of principle, it lacked control over the number of ions that entered the trap, suffered from metastable decay of peptide ions, and had poor ion transmission and mass range. Technological advances to address these issues, and many others, would be required for the Orbitrap mass analyzer to achieve real-world usability.

A key and particularly challenging step in the practical implementation of the Orbitrap analyzer involved the development of an external storage device, later called the C-trap (**Figure 1**), that

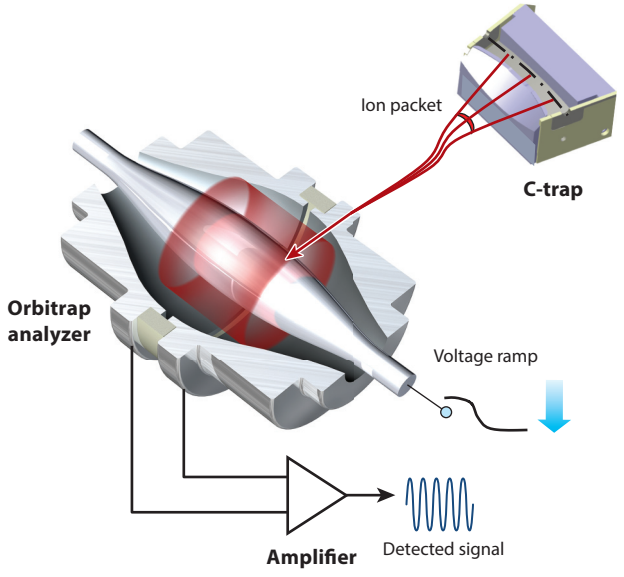

Figure 1

Cross section of the C-trap ion accumulation device and the Orbitrap mass analyzer with an example of an ion trajectory. During the voltage ramp, the ion packets enter the Orbitrap mass analyzer forming rings that induce current which is detected by the amplifier. Reprinted with permission from Thermo Fisher Scientific, Copyright 2015.

ION ACCUMULATION AND THEIR DETECTION IN AN ORBITRAP MASS ANALYZER

Ions enter the radiofrequency (RF)-only bent quadrupole of the C-trap and get stored there as they lose energy in gentle collisions with the bath gas. The RF voltage is then ramped down and a high-voltage pulse is applied across the trap, ejecting ions orthogonally to its curved axis. As the original thread of ions disperses into short packets of different *m/z*, dedicated bent ion optics focuses these packets onto the entrance aperture of the analyzer.

Ion packets enter the Orbitrap analyzer at an offset from its equator and experience strong radial and axial fields. The axial component of the field forces axial acceleration and hence so-called excitation by injection, while the radial component sets ions on a circular orbit around the central electrode. As the voltage on this electrode increases during the injection process, the radius of ion packet rotation gets squeezed down.

Because of the strong dependence of the rotational frequencies on the ion energies, angles, and initial positions, each ion packet soon spreads over the angular coordinate and forms a thin rotating ring. After voltages are stabilized, the differential amplifier detects a current induced by these rings on the split outer electrodes of the trap.

allowed accumulation of ions before injection into the Orbitrap analyzer and hence permitted interfacing the discontinuously operated Orbitrap analyzer to continuous ion sources such as electrosprays (11–13) (see sidebar, Ion Accumulation and Their Detection in an Orbitrap Mass Analyzer). Once successfully coupled with electrospray ionization, complex mixtures could be resolved with the Orbitrap mass analyzer; however, there remained a need to provide control over the size of the ion population to avoid the severe space charge effects known to occur in the external storage device (14).

THE COMMERCIAL RELEASE OF THE FIRST ORBITRAP MASS ANALYZER

By 2005, high-end FT-ICR instrumentation was considered state of the art and was utilized by researchers in the fields of proteomics and metabolomics, among many others. It was obvious that an instrument that offered a compact and easy to use mass analyzer exhibiting performance even remotely similar to that of an FT-ICR coupled to a mass analyzer capable of ion isolation and fragmentation would be embraced by researchers. In this vein, an Orbitrap-based tandem mass spectrometry (MS/MS) instrument would provide the resolution, mass accuracy, and speed desired by the community while eliminating the regular operational maintenance (as well as extensive laboratory space and site preparation) required by FT-ICR systems.

MS/MS: tandem mass spectrometry

HR/AM: high-resolution, accurate mass

LC: liquid chromatography

CID: (ion trap–based) collision-induced dissociation

A logical first step into the world of high-performance MS, where both high mass resolution ion detection and MS/MS analyses are combined, involved the pairing of the Orbitrap mass analyzer with a linear ion trap mass analyzer (15). The high-resolution, accurate mass (HR/AM) detection offered by the Orbitrap mass analyzer was coupled with the sensitive ion detection, precursor isolation, and fragmentation capabilities associated with the linear ion trap mass analyzer. This hybrid instrument architecture capitalized on a full decade of successful use of ion traps in proteomics, and particularly on the development of the linear ion trap mass analyzer. Linear ion traps had quickly become an important analytical tool in the development of the proteomics field, as they provided the speed and sensitivity required for liquid chromatography (LC)–compatible full scan and MS/MS detection of peptides. The linear trap provided very high sensitivity for both MS and MS/MS detection, the ability to perform multiple levels of precursor isolation (MS^n

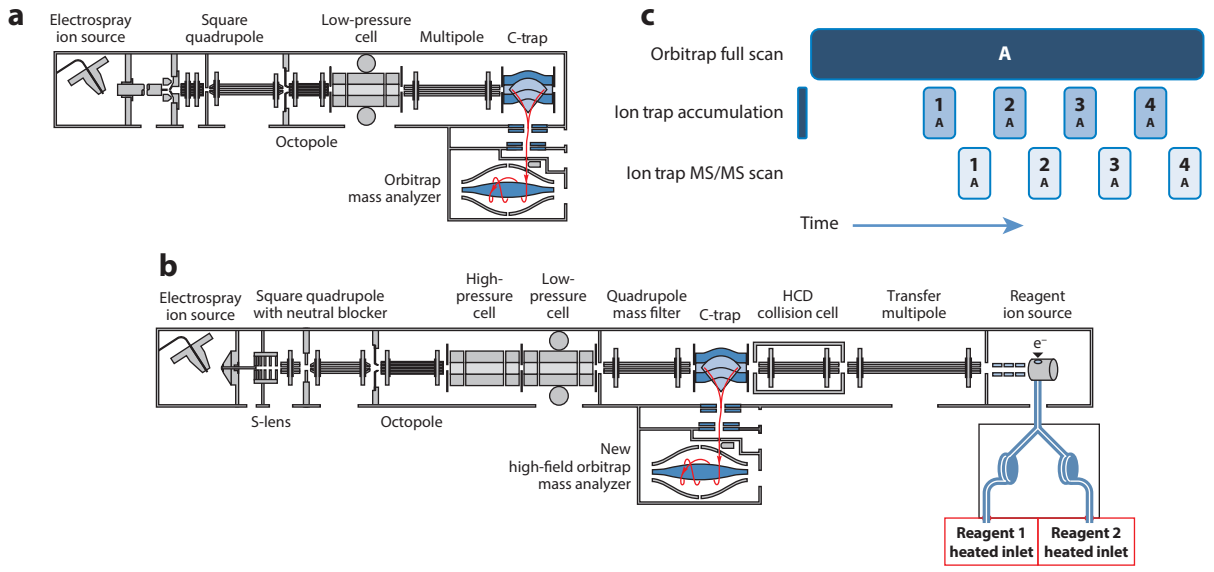

Figure 2

Ion trap/Orbitrap hybrid mass spectrometer architecture and scan execution schema. (*a*) Schematic of the LTQ Orbitrap mass spectrometer with traditional ion trap followed by Orbitrap mass spectrometer architecture. (*b*) Schematic of the Orbitrap Elite mass spectrometer with S-lens, dual-pressure linear ion trap, HCD cell, and high-field Orbitrap mass analyzer. (*c*) Diagram of the preview mode used on hybrid ion trap/Orbitrap systems to improve parallelization and increase scan rates. Reprinted with permission from Thermo Fisher Scientific, Copyright 2015. Abbreviations: HCD, higher energy collision-induced dissociation.

capabilities), and precursor fragmentation [by resonant collision-induced dissociation (CID)] at scan rates sufficiently high to meet the requirements of proteomics experiments.

In 2005, Thermo Electron (now Thermo Fisher Scientific) introduced the LTQ Orbitrap mass spectrometer, which was the first commercial instrument to incorporate an Orbitrap mass analyzer (13). The original design of the LTQ Orbitrap instrument, with the ion trap followed by the Orbitrap mass analyzer (**Figure 2*a***), allowed the flexibility required by researchers. Both MS and MS^n spectra could be recorded by using either the Orbitrap analyzer for highest resolution and mass accuracy or the ion trap analyzer for highest speed and sensitivity. The most commonly employed operation mode for the instrument became acquisition of full scans in the Orbitrap analyzer and data-dependent MS/MS scans in the ion trap analyzer. This mode allowed full utilization of the resolution and mass accuracy for the detection of precursors in complex mixed spectra and the speed and sensitivity for MS/MS spectra on an LC timescale in discovery-based experiments, similar to how some FT-ICR-type instruments are operated with full scans at higher resolution and MS/MS at lower resolution to maximize speed of analyses.

One of the main advantages of HR/AM systems is that they allow accurate charge state and mass determination of multiply charged species in complex mixtures, fulfilling a longstanding need of the proteomics community. With low parts-per-million accurate precursor mass detection, database searching is greatly simplified and improved (16). In discovery-based experiments, HR/AM full scan detection (having resolving powers up to 120,000 FWHM at m/z 400) in the Orbitrap analyzer provides a list of precursors that are then analyzed by MS/MS using CID fragmentation within a single LC run. The LTQ Orbitrap instrument proved valuable in peptide identification almost immediately after its introduction, with scan rates that reached 4–5 Hz in MS/MS with nominal mass detection, or up to 3 Hz MS/MS with accurate mass detection.

AGC: automatic gain control

PTMs: post-translational modifications

HCD: higher-energy collision-induced dissociation

ETD: electron transfer dissociation

QTOF: quadrupole time-of-flight

The LTQ Orbitrap instrument became widely accepted not only in the qualitative fields, but was also found useful for researchers performing quantitative analyses, owing to a variety of key properties and features. The stability of its mass accuracy allowed narrow extraction windows for precursor quantitation. Interference peaks were resolved from analytes of interest by use of high-resolution detection, thus increasing the accuracy of the quantitation. Fast, sensitive MS/MS allowed identity confirmation of quantified analytes, even at low levels.

Quantitative accuracy and dynamic range were ensured using automatic gain control (AGC). AGC of the ion population is a process by which a short prescan in the linear trap is used to determine the ion current within the mass range of interest. This enables storage of a defined number of ions (the AGC target value) for the subsequent analytical scan, eliminating the problems caused by space charging while maintaining a sufficient number of ions to ensure high sensitivity.

The analytical power of mass resolution and accuracy, combined with the built-in flexibility of the instrument, promoted the use of the LTQ Orbitrap platform by a large community of researchers. However, certain limitations of the instrument were apparent. First, the only fragmentation method available was ion trap–based CID, a method that proved powerful for peptide identification but was limited for modified peptides with important post-translational modifications (PTMs) such as phosphorylation and glycosylation. In addition, the practical accurate mass MS/MS scan rate was slow, impeding its utility in experiments requiring online chromatography.

FRAGMENTATION FLEXIBILITY

Subsequent instrument developments were focused on expanding the analytical capabilities for increasingly complex applications, such as the aforementioned identification of post-translationally modified peptides. For more sensitive analysis, the C-trap was improved to provide higher transmission (the LTQ Orbitrap XL mass spectrometer). To obtain structural information not afforded by low-energy CID fragmentation, additional fragmentation techniques were implemented that required adding a new multipole for higher energy collision-induced dissociation (HCD) as well as a newly developed electron transfer dissociation (ETD) reagent ion source (17).

The implementation of HCD in Orbitrap-based instruments was driven by a desire to provide beam-type collisional dissociation similar to that offered in triple quadrupole instruments, as well as in quadrupole time-of-flight (QTOF) mass spectrometers that were at the time the most widely used accurate mass instruments (18). To perform fragmentation with HCD, a gas-filled quadrupole (the HCD cell) was fitted directly after the C-trap (19). This so-called dead end geometry was primarily intended to locate the HCD cell relatively remote from the linear ion trap mass analyzer and thereby reduce gas backstreaming into the linear trap. The geometry also proved to be useful in terms of allowing the instrument to be more compact and providing greater flexibility with regard to operational modes. The HCD cell fragments ions by adjusting the DC offset applied to the rod electrodes to provide the collision energy required to induce fragmentation, in a similar fashion to that of a conventional collision cell (19). Notable benefits of implementation of the HCD cell on the ion trap/Orbitrap hybrid instrument include facilitating the production of immonium ions, de novo sequencing of peptides, and building highly informative fragmentation libraries.

The importance of PTMs in proper biological function including cell signaling, protein structure, and protein stability is well known. MS has played a key role in identifying and quantifying PTMs, particularly in the case where a priori knowledge of the particular PTM was not known (20). Some of the more labile PTMs, such as phosphorylation and glycosylation, are of great interest to scientists; however, they have proved difficult to detect.

ETD, a nonergodic fragmentation technique, was developed in 2004 on stand-alone ion trap mass spectrometers to improve the sequence coverage of proteins and peptides, as well as to

preserve labile-PTM attachment for their identification, localization, and quantification (21, 22). This source was, however, incompatible with Orbitrap-based instruments, as it interfaced with the linear trap at the same position as the Orbitrap was interfaced. The first commercial ETD reagent ion source on an Orbitrap-based system generated fluoranthene reagent anions in a source located behind the HCD cell. The reagent anions passed through the HCD cell and C-trap into the linear ion trap mass analyzer, where reactions with peptide cations took place (17). ETD fragmentation most commonly occurs on the peptide backbone, leaving the PTM(s) intact. As ETD favors precursors with high charge and low *m/z*, more complete fragment ion coverage could be achieved by combining ETD data with CID data. This led to the adoption of ETD not only for PTM identification and unambiguous localization, but also for peptide and protein sequence determination. Owing to the sensitivity of ETD to charge density, an optimized data-dependent decision tree method was developed that routed specific ions to either ETD or CID based on their charge and *m/z* (23).

This initial version of ETD played an increasingly important role in PTM analysis using MS and paved the way to wide adoption of this novel technique in the proteomics community. At the same time, however, there remained significant room for improvement for its practical implementation.

HOW INCREASED SENSITIVITY FUELS THE NEED FOR SPEED

Now that most types of peptides were readily fragmented and identifiable by LC-MS/MS analysis, a major shift in proteomics analyses began to occur. There was a strong desire among researchers to directly analyze complex proteomics samples without extensive prefractionation with techniques such as SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) separation and two-dimensional gel electrophoresis.

Pioneering groups, such as Don Hunt's at the University of Virginia and John Yates' at Scripps Research Institute, had been developing multiple protocols to provide the separations necessary to perform analysis of complex peptide mixtures without extensive offline fractionation (24, 25). However, widespread use of these types of online analyses was hindered by the limited sensitivity and/or slow analytical acquisition rate of then-available HR/AM instruments.

The continued development of mass spectrometers, including Orbitrap-based systems as well as other instrument platforms such as the QTOF, was thus driven by objectives of increasing sensitivity and accelerating scan speed to meet the evolving needs of proteomics researchers. In 2009, the LTQ Orbitrap Velos hybrid mass spectrometer was released with new capabilities aimed directly at achieving these objectives (26). Increased sensitivity (3–5-fold in full scan mode and up to 10-fold in MS/MS mode) was partially effected by substitution of the capillary-skimmer interface with a novel stacked ring ion guide, known as the S-lens (27). This improvement in the ion source optics paralleled similar developments in the mass spectrometer industry, such as the implementation of ion funnel technology developed at PNNL (28, 29), Q-jet™ at Sciex (30), and later Stepwave™ at Waters (31).

In addition to the S-lens, the LTQ Orbitrap Velos incorporated a novel dual-pressure ion trap mass analyzer, consisting of a high-pressure cell optimized for isolating and fragmenting ions and a low-pressure cell optimized for accelerated ion analysis. This ion trap configuration enabled faster acquisition rates without diminishing spectral quality (27). The combination of the S-lens with the dual-pressure ion trap improved the system sensitivity by transferring more ions into the system and then more effectively and rapidly manipulating them. Additionally, ion injection times for MS/MS were now being predicted using the intensity of the ions detected in the preceding full scans (known as predictive AGC or pAGC), instead of performing distinct

prescans for each precursor, thereby saving a significant amount of time per analysis cycle. In the aggregate, these technological advances routinely enabled ion trap MS/MS acquisition rates of up to 10 Hz and made proteomics analysis of nonfractionated samples an everyday reality (32). Significant improvements to the ion transfer efficiency inside the mass spectrometer, coupled with the brighter ion source, also allowed for much more sensitive HCD HR/AM spectra at 4–5 Hz.

TMT: tandem mass tags

SILAC: stable isotope labeling by amino acids in cell culture

TRANSITIONING TO QUALITATIVE AND QUANTITIVE ANALYSIS

As identification rates improved, a new trend of seeking quantitative information from experiments that were previously solely qualitative evolved. This trend was driven mainly by the realization that many biological changes were not simply on/off changes, but rather more subtle changes in the abundance of a protein, PTM, or groups of proteins/PTMs. For MS-based proteomics to excel as a technique for the successful identification and characterization of biologically meaningful changes, it needed to be able to identify changes in protein abundance. The number of samples that needed to be analyzed for a given experiment increased dramatically. Technical and biological replicates required for each study increased with the need to identify statistically significant changes between samples. To combat the throughput issues that this created for most laboratories, a variety of quantitative approaches were developed, including isobaric tagging [i.e., isobaric Tags for Relative and Absolute Quantitation (iTRAQ^{TM}) and Tandem Mass Tags (TMT) labeling reagents] and stable isotope labeling by amino acids in cell culture (SILAC) that allow direct analysis of multiple samples in a single run (33–36).

Accurate SILAC quantitation requires very high-resolution analysis. As precursor peak areas of light and heavy isotopically labeled peptides are used for quantitation, analysis of complex samples is particularly at risk for interference by isobaric peaks when resolution is insufficient. The tension between the need for increased resolving power (hence longer MS analysis) and the need for more MS/MS scans was addressed in the LTQ Orbitrap Velos instrument by the increasingly parallel operation of the linear trap and Orbitrap analyzers.

At this time, successful quantitative analysis of isobarically tagged multiplexed samples relied on a collision cell. In this application, one of the main advantages of HCD fragmentation is that beam-type fragmentation allows the collection of fragments with a lower mass cutoff than resonant CID fragmentation typical for ion trap mass analyzers, preserving the required detection of the reporter ions in the low mass region of the MS/MS spectra. To improve sensitivity of HCD MS/MS spectra on the LTQ Orbitrap Velos instrument, an axial field gradient or drag field was applied to the HCD cell to help eject more ions out of the cell, allowing more sensitive Orbitrap detection of the peptide fragment and quantitative reporter ions. Improved HCD tuning was achieved by locating the HCD cell closer to the C-trap and simplifying their interface such that they were separated solely by a diaphragm. With these technological advances, quantitation using a trapping analyzer quickly became a reality, ultimately encouraging development of other types of Orbitrap hybrids.

FROM RESEARCH TO ROUTINE: ORBITRAP TECHNOLOGY IN ROUTINE LABORATORIES

Although the advantages of HR/AM MS systems in fields such as proteomics for qualitative and quantitative analysis were widely recognized, the cost and complexity of the Orbitrap hybrid platform hindered its adoption by routine laboratories (such as food safety and toxicology) in spite of a growing desire to take advantage of the added information afforded by HR/AM analysis in these laboratories. The development of the Exactive instrument platform (37) was intended to

make HR/AM technology accessible to routine laboratories and other users by reducing the cost and complexity of Orbitrap-based instrumentation. The Exactive instrument utilized a stand-alone Orbitrap analyzer (omitting the ion trap mass analyzer present in the hybrid platforms), offering the ability to design the instrument as a compact bench-top unit. The Exactive was capable of full scan detection and HCD without precursor isolation. This mode of analysis, referred to as all-ion fragmentation in connection with its implementation in the Exactive instrument, is similar to analogous techniques available on instruments sold by Sciex and Waters (the MS^{ALL} and MS^{E}, respectively). The omission of the ion trap mass analyzer in the Exactive mass spectrometer necessitated the development of techniques for an AGC mechanism that used only Orbitrap detection. This approach to AGC was later augmented further by adding a discrete charge detector.

The introduction of the Exactive instrument facilitated HR/AM screening of known and unknown compounds with extremely high selectivity (less than 5 ppm). Full scan MS screening on any HR/AM instrumentation also offers the opportunity to allow retrospective data analysis based on a posteriori hypothesis of additional compounds of interest, owing to the full scan data acquisition rather than strictly targeting specific ions of interest as is done with standard triple quadrupole analyses. With high intrascan dynamic range (four orders of magnitude) and fast polarity switching (one positive and on negative HR scan per second), the Exactive mass spectrometer addressed a broad range of applications from discovery to quantitation to elemental composition determinations.

The subsequently developed Q Exactive instrument permitted precursor ion isolation on an Exactive-type mass spectrometer (38) (**Figure 3*a***). A mass filtering quadrupole was utilized

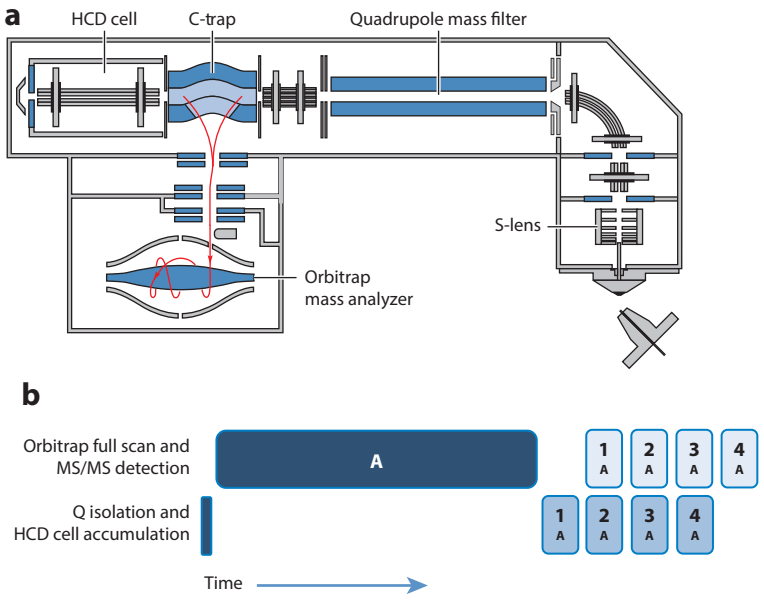

Figure 3

Quadrupole/Orbitrap hybrid mass spectrometer architecture and scan execution schema. (*a*) Schematic of the Q Exactive mass spectrometer. (*b*) Diagram of the parallel ion accumulation and detection implementation with Q Exactive mass spectrometers. Reprinted with permission by Thermo Fisher Scientific, Copyright 2015. Abbreviations: HCD, higher energy collision-induced dissociation; MS/MS; tandem mass spectrometry.

for isolation of precursors and an Orbitrap analyzer employed for detection of full scans and MS/MS spectra, using an HCD cell for fragmentation. By this time, ultrahigh performance liquid chromatography (UHPLC) had become common at both conventional and nano flow rates, and thus improving acquisition rates were becoming increasingly important, particularly to ensure sufficient numbers of points across the LC peak for quantitation. The increased speed of nominal-mass MS/MS provided by the LTQ Velos had not yet been followed by a similar increase of accurate mass MS/MS speed; this was addressed by the Q Exactive instrument.

UHPLC: ultrahigh performance liquid chromatography

eFT: enhanced Fourier transform

The ability to acquire MS/MS spectra at relatively high resolving powers, but with reduced transient duration and hence faster acquisition rates, was achieved with the development of an advanced signal processing technique for transforming the transient detection signal produced by the Orbitrap mass analyzer, termed enhanced Fourier transform (eFT). This technique increases the spectral resolution at any given transient duration (39). eFT incorporates information about phases of ion oscillations that are precisely defined as a result of the excitation mechanism unique to the Orbitrap analyzer. As ions are injected from the C-trap into the Orbitrap analyzer in very short packets, they enter the analyzer at an offset from its equator, so that the electric field at this point is directed not only radially for orbital trapping but also axially, thus initiating axial oscillations without any need for additional excitation. As the flight time from the C-trap to the analyzer is inversely proportional to $(m/z)^{1/2}$ in the same way as the period of axial oscillations, this external portion of ion trajectory is equivalent to a certain, almost m/z-independent share of an oscillation period and could be regarded as a phase shift. By correcting for this shift, it is possible to create an absorption spectrum with peak width reduced by one half, as if the transient was mirrored relative to this virtual common starting time for all ions and thus doubled in length.

The combination of the S-lens, fast and efficient quadrupole isolation with HCD fragmentation, and Orbitrap detection with eFT provided improved data quality and acquisition rates. Permitting full MS/MS parallelization where the HCD cell or the C-trap is filled with ions while the previous MS/MS detection cycle is ongoing, the system achieves a very high duty cycle (**Figure 3*b***). By allocating most of the analysis time for accumulating ions, the sensitivity is maximized, alleviating the impact that low ion fluxes could have on acquisition speed and spectral quality. High-resolution MS/MS could now be acquired at a rate of up to 12 Hz, thus avoiding the problem of low speed for accurate mass MS/MS and representing an advance in Orbitrap system architecture and performance (38).

HIGH-SPEED ANALYSIS WITH THE HIGH-FIELD ORBITRAP ANALYZER

The compact, high-field version of the Orbitrap analyzer was introduced in 2011 as a part of the Orbitrap Elite instrument (40) (**Figure 2*b***) and was later utilized in the Q Exactive HF and Orbitrap Fusion instruments (**Figure 4*a***). This advanced analyzer effectively doubles the operating frequency of the first commercial version of the Orbitrap mass analyzer. Additionally, the eFT technique described above further doubles the resolving power to 240,000 at m/z 400 for a 768-ms transient, yielding nearly a fourfold increase in resolving power for the same transient length relative to the earlier Orbitrap design. These developments required modifications to the adjacent ion optics, preamplifiers, and machining accuracy of the Orbitrap electrodes (41). Other developments were incorporated in the Orbitrap Elite (and later generation) instruments with an objective of improving the robustness of the ion transfer optics and MS/MS acquisition rates of the dual cell linear ion.

The increased acquisition rate/resolving power of the high-field Orbitrap mass analyzer provided benefits to researchers for a variety of applications. Significantly higher resolution could be obtained from the same transient length, which is particularly advantageous in quantitative

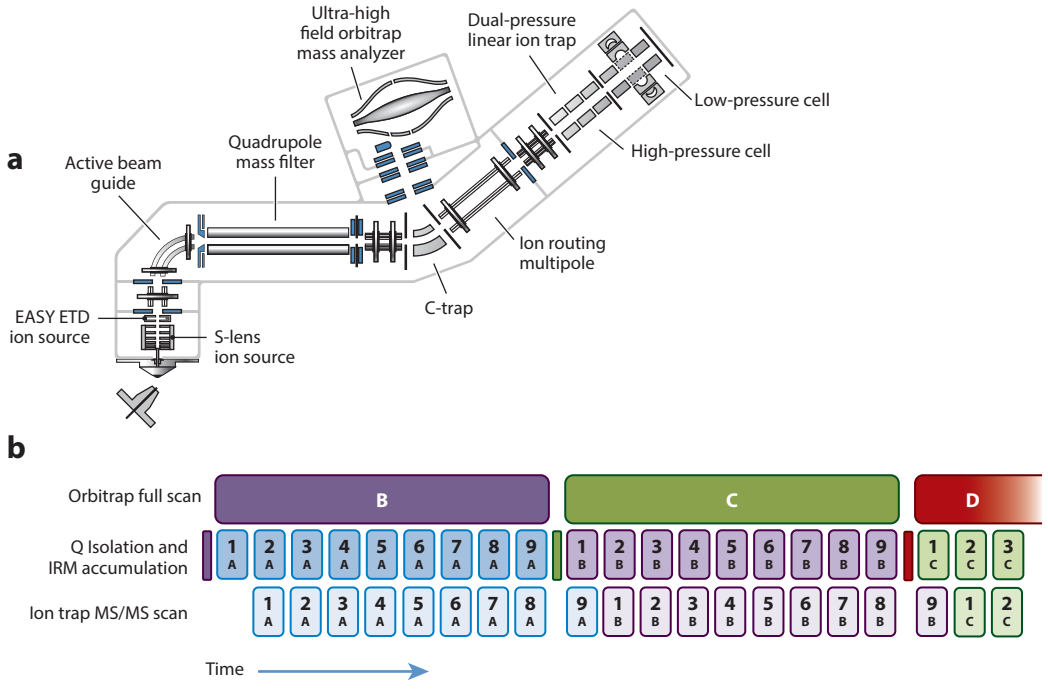

Figure 4

Quadrupole/Orbitrap/ion trap tribrid mass spectrometer architecture and scan execution schema. (*a*) Schematic of the Orbitrap Fusion mass spectrometer. (*b*) Diagram of the parallel ion accumulation and detection implementation with the Orbitrap Fusion mass spectrometer. Reprinted with permission from Thermo Fisher Scientific, Copyright 2015. Abbreviations: ETD, electron transfer dissociation; IRM, ion routing multipole; MS/MS, tandem mass spectrometry.

experiments such as SILAC analysis of complex samples where higher resolution analysis is desired but was not previously accessible due to the associated lower acquisition rates and consequently the loss of peptide identifications. Not wanting to sacrifice the MS/MS rate in such experiments where both resolution and the acquisition rate are important, a preview mode was implemented on the first hybrid Orbitrap/ion trap systems to allow high-resolution MS analysis and, to a degree, parallelize the data-dependent MS/MS analyses (**Figure 2*c***). This preview mode generates its list of precursors to analyze by data-dependent MS/MS at slightly lower resolution (15 K for the LTQ Orbitrap mass spectrometer and 60 K for Orbitrap Elite mass spectrometer); the Orbitrap analyzer continues to acquire and record the full, high-resolution scan while the data-dependent MS/MS spectra are being acquired in the ion trap, allowing for significantly reduced acquisition cycles, although not achieving complete parallelization. Alternatively, the high-field Orbitrap analyzer can be operated at higher acquisition rates for the same resolution compared to earlier Orbitrap-based mass spectrometer systems, ultimately achieving a faster acquisition cycle and increasing the number of MS/MS analyses in a given run, providing increased numbers of identifications and deeper penetration into the sample.

IMPROVING INTACT PROTEIN AND TOP-DOWN ANALYSIS

There is a growing interest among researchers in analyzing proteins without or with only minimal digestion to retain connectivity of PTMs on a single protein and also to determine

sequence isoforms more readily. Much of this work, pioneered by the research groups led by Fred McLafferty and Neil Kelleher, was done on FT-ICR systems, taking advantage of the extremely high-resolution capabilities (42, 43).

On earlier Orbitrap-based mass spectrometers, performing intact protein experiments was rendered difficult by the short lifetime of large ions in the Orbitrap mass analyzer and the delayed start of the detection process. Collisions of unstable ions with gaseous species would quickly damp the signal and hinder the detection in the Orbitrap analyzer. The LTQ Orbitrap Velos instrument had reduced residual pressure in the Orbitrap analyzer that significantly improved the analysis of intact proteins that are particularly susceptible to collisions. Intact antibodies had been studied with the earlier Orbitrap-based mass spectrometers (44, 45); however, with these improvements to the LTQ Orbitrap Velos mass spectrometer, routine and reliable detection of intact antibodies was greatly facilitated.

For high quality MS and MS/MS spectra of proteins, multiple microscans (averaging of the transients) are typically required due to the limited ion current that otherwise, may result in a low signal-to-noise ratio. This limited ion current is a result of a single protein being split between many charge states, the presence of many modified species, and/or the sheer number of fragment ions that can be generated from an intact protein. Owing to its ability to acquire spectra at accelerated acquisition speed, the high-field Orbitrap mass analyzer extended the abilities of Orbitrap systems to perform top-down experiments with HR/AM MS and MS/MS spectra on an LC-timescale (46). The increased speed of detection also allowed for more opportunities to perform CID, HCD, and/or ETD fragmentations, which provide complementary information for more complete characterization (46).

To better meet the requirements necessary for the analysis of intact antibodies, antibody-drug conjugates, large protein complexes, and native proteins, an extended mass range Orbitrap instrument was developed with improved sensitivity to these analytes (47–50). These mass spectrometers have been optimized for improved transmission of higher mass-to-charge-ratio ions, enabling detection up to *m/z* 20,000. Additionally, the signal-to-noise ratio is improved as a result of the adjustable HCD cell pressure, access to short transients, and controls for easy optimization of experimental conditions.

CAPABILITIES FOR SMALL MOLECULE ANALYSES

Analyses of small molecules present unique challenges relative to proteomics experiments. Identifying, quantifying, and characterizing the structure of small molecules often requires extremely high mass accuracy and resolution and/or multiple stages of MS^n. A wide variety of compounds are now routinely analyzed by Orbitrap-based instruments, typically coupled with UHPLC. The role of Orbitrap-based analysis is growing rapidly in a variety of applications, including those of food safety and environmental analysis (51–53), metabolite and metabolomics analysis (54–60), clinical analysis (61–63), bioanalysis (64, 65), doping control (66–68), and lipidomics (69–72).

When detecting analytes such as drugs and their metabolites in biological matrices, endogenous components can often mask the detection of the target analytes. HR/AM analyses permit narrow mass extraction windows that reduce or eliminate background chemical noise and significantly improve the detection limits for metabolite analysis (73, 74). Combining targeted analysis with unbiased metabolite profiling is achievable through the use of HR/AM data acquisition, where theoretical masses are used for the generation of extracted ion chromatograms. The detection of accurate mass information for all ions in the full scan spectrum, providing a degree of specificity equal to most SRM-based assays, is the principal advantage of the HR/AM MS approach (75). This technique has been used in an in vitro absorption, distribution, metabolism, and excretion

workflow of cassette incubation for as many as 32 compounds, followed by quantitative bioanalysis using full scan acquisition (64). Targeting of compounds can also now be achieved on the fly for a variety of analyte groups, including those containing metals (76) or those with distinct isotopic patterns such as sulfur- and bromine-containing (77, 78).

For small molecule analyses including complex metabolomics studies, HR/AM MS can identify analytes through elemental composition determination via isotopic fine structure examination using exact mass and isotopic patterns. Fine structure determination is useful for both compound confirmation and identifying unknowns and requires ultrahigh resolving power to detect peaks with mass differences of as little as 3–6 mDa, which accurately provide the relative isotopic abundances ^{13}C, ^{15}N, ^{18}O, and ^{34}S. In addition to more traditional small molecule characterization, the high-field Orbitrap analyzer has also opened up intriguing applications requiring ultrahigh resolution, previously amenable only to high-field FT-ICR MS instrumentation, such as petroleomics (79).

Structural elucidation and identification has been successfully performed using Orbitrap analyzers (54, 55). High mass accuracy fragment ions can be more readily assigned and correctly annotated (80) making HR/AM data particularly useful for nontargeted metabolite and biomarker identification with simultaneous targeted bioanalytical quantitation in a single run (37). To obtain more complete structural information, multiple levels of fragmentation (MS^n) possible with the ion trap/Orbitrap hybrid instruments are often required to break enough bonds sequentially to fully elucidate the structure.

TOWARD ULTIMATE FLEXIBILITY IN FRAGMENTATION AND EXPERIMENTAL DESIGN

The Orbitrap Fusion instrument, incorporating a quadrupole mass filter and Orbitrap and linear ion trap mass analyzers, combined advanced ion trap Orbitrap hybrid technology with the quadrupole Orbitrap hybrid systems (81) (**Figure 4*a***). This architecture enabled significant performance improvements including achieving 500,000 resolving power at *m/z* 200. With three mass analyzers, operation can be fully parallelized, maximizing the use of the ion current (**Figure 4*b***). The instrument architecture facilitates the realization and rapid execution of complex modes of analysis, due to its characteristic ability to concurrently isolate ions with one analyzer and separately detect ions in the two remaining analyzers.

As an illustration of the flexibility of the Orbitrap Fusion system, isolation can be performed with either the quadrupole mass filter or the ion trap mass analyzer, whereas fragment ions can be generated by HCD, CID, ETD, or the novel EThcD fragmentation type (82) at any level of MS^n. Moreover, these precursor and fragment ions can be detected in either the Orbitrap or ion trap analyzer. The expanded flexibility of analysis allows methods to be adapted to novel arrangements (83) and to answer very challenging structural questions.

Prior to introduction of the Orbitrap Fusion instrument, data-dependent experiments usually followed a so-called Top*N* approach, where *N* qualifying precursors were chosen for fragmentation according to their intensity in descending order. In the Orbitrap Fusion instrument, choosing precursors to target for data-dependent MS^n is no longer restricted to most-intense Top*N*, but additionally allows selection of the targeted precursors according to high or low *m/z* or charge state. In addition to a Top*N* mode where the users define the maximum number of MS/MS to perform per cycle, a more flexible mode, known as Top Speed, was developed that allows a user to define the cycle time (minimum frequency of MS scans). The system will then perform the maximal number of accompanying MS/MS scans without exceeding the requested master scan spacing, which is particularly important when precursor quantitation requires a minimum number of points across the chromatographic peak.

SPS: synchronous precursor selection

DIA: data-independent acquisition

msxDIA: multiplexed DIA

HCD fragmentation often generates more fragment ions (19) relative to resonant CID fragmentation. When HCD is used for small molecule structural characterization, more fragment ion information can be gleaned with fewer stages of MS^n analyses. Detection of HCD fragment ions can be, for the first time in a hybrid ion trap-Orbitrap instrument, performed in the ion trap mass analyzer (for the highest sensitivity and degree of parallelization) or in the Orbitrap mass analyzer (for the highest resolution) at any level of MS^n, improving HR/AM analyses for experiments such as small molecule characterization, glycomics, metabolomics, and lipidomics profiling.

The Orbitrap Fusion instrument incorporates a novel ETD reagent ion source, utilizing Townsend discharge for ionization of the reagent molecules, which significantly reduces maintenance and tuning requirements (84) relative to the earlier filament-based ETD ionization source. The inclusion of a novel option for preferential selection of higher charge ions with lower m/z allows the Orbitrap Fusion instrument to target ions, in a data-dependent fashion, known to be more susceptible to ETD fragmentation, thereby potentially increasing the identification rate (83).

New techniques to meet the demands for fast but also accurate TMT analyses also led to the development of synchronous precursor selection (SPS), an isolation mode that permits multiple fragment ions to be simultaneously isolated for a third stage of MS^n analyses using multi-notch isolation in the ion trap mass analyzer (85). This SPS isolation mode significantly increases the sensitivity of the selective and accurate TMT MS^3 approach (86). With the ability to perform multiple isolations and detections in parallel, highly accurate SPS-TMT-MS^3 analyses can be done at a speed that rivals previous MS/MS-type TMT experiments and delivers significantly improved quantitation accuracy.

TIME FOR DATA INDEPENDENCE: THE SPEED AND RESOLUTION NECESSARY FOR COMPLETE AND SENSITIVE MS^2 ANALYSIS

Interest in data-independent acquisition (DIA) (87), which has been implemented in various forms by other mass spectrometer vendors [e.g., as the SWATH™ acquisition technique available on instruments by Sciex (88)], has been growing rapidly for both the identification and quantification of peptides detected in a complex mixture. In DIA experiments, MS/MS analyses are performed over the entire defined mass range in contrast to the conventional data-dependent acquisition approaches that trigger MS/MS only when ions of specific intensity, charge state, etc., are detected in the full scan. Cycle times to perform MS/MS over an entire mass range of interest vary greatly depending on the acquisition speed and the isolation window widths of the MS/MS spectra. DIA approaches have been developed on each of the Orbitrap mass spectrometer platforms to take advantage of their specific architectures.

Q Exactive–based DIA experiments use fast acquisition speeds with sensitive detection to allow narrow MS/MS isolation windows over a wide mass range while maintaining reasonable cycle times with high resolution and mass accuracy. Narrow DIA isolation windows increase the selectivity and sensitivity of analyses relative to wider isolation windows. Multiplexed DIA (msxDIA) experiments, as developed by Egertson et al. (89), further increase the acquisition rate of DIA experiments. In msxDIA experiments, multiple packets of ions from across the mass range are sequentially isolated with the quadrupole and are fragmented and stored in the HCD cell. Once the mixed population of ions is built up, they are analyzed in combination in a single Orbitrap detection event. Multiplexing ions into a single spectrum is most advantageous when the combined injection times does not exceed the detection time. Ultimately, multiplexing is a means of increasing the effective acquisition rate while maintaining narrow isolation windows and is also being used on the Orbitrap-based platforms to improve targeted quantitation experiments in addition to the data-independent qual/quan msxDIA experiments (89, 90). Some DIA modes depend strongly on

isolation windows being sharply defined, particularly those with overlapping windows (89); this requirement was addressed later by the Q Exactive Plus instrument (2013). Development in the area of DIA, including methods such as WiSIM-DIA on the Orbitrap Fusion mass spectrometer (91), continues to be driven by researchers interested in reproducibly identifying and quantifying species across multiple samples.

FUTURE ISSUES

As we have discussed here, advances in Orbitrap technology (both hardware and software) have been driven by the needs and desires of the individuals that are using the mass spectrometers as tools to solve their analytical problems and advance scientific discovery. Nearly every new feature or performance improvement not only enhances a current experiment but also suggests a potential new MS application, some of which require further technological advances, continually promoting the instrument development process. Potential future improvements for the technology cover a broad range of possibilities (some yet to be proven) and include the following:

1. Continuous improvement of Orbitrap manufacturing technology enables higher and higher levels of resolving powers, as illustrated by achieving greater than 1,000,000 resolving power on an Orbitrap analyzer (92). Previously, such values were easy to reach on FT-ICR instruments but not on electrostatic analyzers. Such capability is expected to drive the development of future instruments, creating mass spectrometers that can be routinely used in extremely demanding applications such as petroleomics (79), element counting (93), NeuCode quantitation (94), and others.
2. Following the example of TOF technology (95), Orbitrap technology made the first inroads into MS of protein complexes (48), and even viruses (96), and provided considerably higher resolving power and sensitivity. Further progress will be associated with mass selection capability at high *m/z* and more sophisticated techniques for top-down analysis of native protein complexes (97).
3. Signal processing methods promise not only better quantitation capabilities of Orbitrap MS but also resolving powers beyond the limit of Fourier transformation. The latter promise comes with a possible limitation that such so-called super resolution may be achievable only at relatively high signal-to-noise ratios (e.g., 10 s to 100 s, depending on the gain required and local peak density).
4. Expansion of the menu of ion sources toward, for example, electron impact, inductively coupled plasma, or glow discharge types paves way for especially fascinating capabilities for a multitude of new analytical applications.

DISCLOSURE STATEMENT

The authors declare the following competing financial interest(s): The authors are employees of Thermo Fisher Scientific, the corporation that produces Orbitrap mass spectrometers. Beyond this, the authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We thank members of our analytical community that drive the development of new MS technology. We thank M. Senko, C. Katz, and V. Zabrouskov for their critical review of the manuscript.

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Annual Review of
Analytical Chemistry

Volume 8, 2015

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Errata

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