FT-ICR Theory – Ion Cyclotron Motion

- Inward directed Lorentz force causes ions to move in circular orbits about the magnetic field axis

FT-ICR Theory – Ion Cyclotron Motion

Ion cyclotron motion. Ions rotate in a plane perpendicular to the direction of a spatially uniform magnetic field,

Note that positive and negative ions orbit in opposite senses.
FT-ICR Theory – Ion Cyclotron Motion

\[ \omega_c = \frac{qB}{m} \]
Once we make an ion, we move it into the center of the Magnet. Then, we trap it before it can escape.

“Gate” shut before the ion escapes

FT-ICR Theory - Ion Trapping
FT-ICR Theory - Ion Trapping

Magnetic Field (B)

Axial Position
FT-ICR Theory - Ion Trapping
FT-ICR Theory – Combined Ion Motion

\( v_m = \text{magnetron motion} \)
\( v_c = \text{cyclotron motion} \)
\( v_T = \text{trapping oscillations} \)

FT-ICR Theory - Excitation
FT-ICR Theory - Excitation

X

Y

Z or B°

Amplitude

Time

Excitation Electrodes
FT-ICR Theory

Excitation

Alan G. Marshall, Christopher L. Hendrickson, and George S. Jackson

Encyclopedia of Analytical Chemistry,
R.A. Meyers (Ed.), John Wiley & Sons Ltd, Chichester, 2000, pp. 11694–11728
SWIFT Excitation

Single Notch SWIFT Event (MS/MS)

Data Count Affects Resolution (Limited to < 512K)

Freq Cutoff

100%

Power

Start Frequency

End Frequency

Bandwidth

Frequency

SWIFT Excitation,

Excitation Mode: MS/MS

Frequency Data

Power

End Frequency

Start Frequency

Freq Cutoff

Data Count Affects Resolution (Limited to < 512K)

SWIFT Excitation

Single Notch SWIFT Event (MS/MS)
SWIFT Excitation

![Power vs Frequency Graph]

IFT

[Signal Waveform]
FT-ICR Theory - Excitation
On-the-fly SWIFT isolation of a single Isotope of Bovine Ubiquitin

M+4 Isotope

m/z

11+ 10+ 9+ 8+ 778.5 779.0 779.5 780.0 780.5 781

700 800 900 1000 1100 1200 1300 1400 1500

m/z
FT-ICR Theory - Detection
Multiplex Detection in FT-ICR

Time Domain Transient

Differential Amplifier
Multiplex Detection in FT-ICR

![Differential Amplifier Diagram](image.png)

**Time Domain Transient**

![Time Domain Transient Graph](graph.png)

*Time (ms)*

*Image Current*
Signal Apodization in FT MS

\[ f(t) = D(t) = \begin{cases} 1 & t \leq T \\ 0 & t > T \end{cases} \]

\[ A(v) = T \text{sinc} 2\nu T \]
\[ \Delta \nu_{1/2} = 0.6/T \]
\[ S = -22\% \]

\[ f(t) = 2D(t)[1 - t/T] - D(2t)[1 - 2t/T] \]

\[ A(v) = T [\text{sinc}^2 \nu T - 0.25\text{sinc}^2 \nu T/2] \]
\[ \Delta \nu_{1/2} = 0.80/T \]
\[ S = -14\% \]

\[ f(t) = D(t)[1 - t/T] \]

\[ A(v) = 0.5 T [\text{sinc}^2 \nu T] \]
\[ \Delta \nu_{1/2} = 0.91/T \]
\[ S = +5.2\% \]

\[ f(t) = D(t)[0.54 + 0.46\cos \pi t/T] \]

\[ A(v) = [0.27/\pi \nu + 0.92\nu T^2/\pi(1 - 4\nu^2 T^2)] \sin 2\pi \nu T \]
\[ \Delta \nu_{1/2} = 0.9/T \]
\[ S = -0.8\% \]

\[ f(t) = D(t)[1 - (t/T)^2]^2 \]

\[ A(v) = T \pi^{1/2}(\pi \nu T)^{-5/2} J_{5/2}(2\pi \nu T) \]
\[ \Delta \nu_{1/2} = 1.0/T \]
\[ S = -3.2\% \]
Fourier Transforms in FT-ICR

Differential Amplifier

Time Domain Transient

Frequency Spectrum

Fourier Transform

Image Current

Frequency (kHz)

Time (ms)

0 50 100 150 200 250 300

0 0.01 0.02 0.03 0.04 0.05

-0.01 -0.02 -0.03 -0.04 -0.05

0 100 200 300 400 500 600 700 800

0 100 200 300 400 500 600 700 800
Mass Calibration in FT-ICR

Frequency Spectrum

Mass Spectrum

\[
\frac{m}{z} = \frac{A}{f} + \frac{BV_t}{f^2}
\]
Space Charge and Resolution in FT-ICR

LOW ION DENSITY

HIGH ION DENSITY
Effect of Magnetic Field Strength

- Resolving Power
- Highest Non-Coalesced Mass
- Scan Speed (LC/MS)
- Axialization Efficiency

Field Strength (Tesla)

- Number of Ions
- Trapped Ion Upper Mass Limit
- 2D-FT Resolving Power
- Ion Trapping Time
- Ion Energy

Field Strength (Tesla)
FT-ICR Experiment - Event Sequences

- Use a single mass analyzer but separate the mass analysis and ion isolation events in time
- Can perform many successive stages of MS ($\text{MS}^n$)

**Event Sequence**

1. Ionization
2. Ion Transfer / Ion Trapping
3. Parent Ion Isolation
4. Parent Ion Fragmentation
5. Daughter Ion Detection
Ultra-high Resolving Power

Peak Capacity = \frac{(m/z)_{\text{max}} - (m/z)_{\text{min}}}{\Delta m_{50\%}}
<table>
<thead>
<tr>
<th>Separation Method</th>
<th>Maximum # of Components</th>
<th>Maximum Peak Capacity</th>
<th>Theoretical Plates</th>
</tr>
</thead>
<tbody>
<tr>
<td>HP-TLC</td>
<td>6</td>
<td>25</td>
<td>1,000</td>
</tr>
<tr>
<td>Isocratic LC</td>
<td>12</td>
<td>100</td>
<td>15,000</td>
</tr>
<tr>
<td>Gradient LC</td>
<td>17</td>
<td>200</td>
<td>60,000</td>
</tr>
<tr>
<td>HPLC</td>
<td>37</td>
<td>1,000</td>
<td>1,500,000</td>
</tr>
<tr>
<td>CE</td>
<td>37</td>
<td>1,000</td>
<td>1,500,000</td>
</tr>
<tr>
<td>Open Tubular GC</td>
<td>37</td>
<td>1,000</td>
<td>1,500,000</td>
</tr>
<tr>
<td>ESI FT-ICR MS</td>
<td>525</td>
<td>200,000</td>
<td>60,000,000,000,000</td>
</tr>
</tbody>
</table>

\[ \frac{m}{\Delta m_{50\%}} > 200,000 \]

\[ 200 < m/z < 1,000 \]

\[ m_{\text{average}} +/- 0.25 \text{ Da} \]

Skip Prior Chemical Separation and Identify Components by MS!
17,000+ Compositional Distinct Components Resolved by High Resolution 9.4 Tesla Electrospray FT-ICR MS

Negative Ion ESI Mass Spectrum

Positive Ion ESI Mass Spectrum

6,118 resolved components

11,127 resolved components

m/z

Figure 3.1. Combined positive and negative electrospray ionization 9.4-T Fourier transform ion cyclotron resonance mass spectra of a crude oil. Average mass resolving power, \( m/\Delta m \), is \( \sim 350,000 \), allowing for resolution and identification of thousands of basic (right) and acidic (left) species. The 11,127 peaks (right) represent the most complex chemical mixture ever resolved and identified in a single mass spectrum.
Figure 3.11. Negative-ion ESI selective ion accumulation 9.4-T FT-ICR mass spectrum of acidic asphaltenes. Note the resolution of 55 peaks at a single nominal mass.

Currently, detailed chemical composition (class, type, and carbon number) of samples of such complexity is accessible only by FT-ICR MS.

2.6. EI, FD, and APPI for Access to Nonpolars

The success of the first ESI FT-ICR MS analysis of crude oil led to the rapid expansion of the technique to other petroleum-derived materials, such as coal, humic and fulvic acids. However, due to the selectivity of ESI for only the most polar species, other ionization methods are necessary to extend the wealth of compositional detail provided by FT-ICR MS to nonpolar species. To that end, we have recently modified our current instruments to accept commercial electron ionization, atmospheric pressure photoionization and field desorption ion sources. Other researchers have investigated thermal desorption probes coupled with electron ionization or metal complexation to gain access to the non-polars.

EI FT-ICR MS relies on thermal desorption of the sample in an inert heated inlet system prior to ionization. As a result, EI FT-ICR MS is not well suited for analysis of extremely heavy materials such as resids. The operating temperature limit of the oven and thermal stability of the inert inlet coatings prevent operation above 400°C. However, the technique is well suited for the analysis of light to moderately heavy distillates that may be lost to volatilization in FD analysis.

55 Elemental Compositions at a Single Nominal Mass

9.4 T ESI FT-ICR MS

m/Δm_{50%} = 350,000

Acidic Asphaltenes (Extra-Heavy Crude)

Fluorinated Polymer (Internal Calibrant)

m/z

629.00 629.10 629.20 629.30 629.40 629.50 629.60 629.70
Isotopic Fine Structure

Resolving power

\[ \frac{M}{\Delta M_{50\%}} = 8,000,000 \]

Bovine Ubiquitin
\[ C_{378}H_{629}N_{105}O_{118}S_{1} \]
\[ MW_{\text{mono}} = 8559.61581 \text{ Da} \]

Recombinant Monoclonal Antibody (IgG1k)

C\textsubscript{6,528}H\textsubscript{10,088}N\textsubscript{1,728}O\textsubscript{2,098}S\textsubscript{44}

1,324 Amino Acids and Multiple Glycoforms

Major mAb N-Glycans

- Fucose (Fuc)
- Mannose (Man)
- Galactose (Gal)
- N-AcetylGlucosamine (GlcNAc)
Unit Mass Baseline Resolution for an Intact 148 kDa Therapeutic Monoclonal Antibody by FT-ICR Mass Spectrometry

Heated Metal Capillary: 75 V, 7.5 A
Skimmer: 56 V

Quadrupole Isolation

(+) ESI 9.4 T FT-ICR MS

IgG1k Monoclonal Antibody

2,200 2,400 2,600 2,800 3,000 3,200
m/z
Unit Mass Baseline Resolution for an Intact 148 kDa Therapeutic Monoclonal Antibody by FT-ICR Mass Spectrometry

Quadrupole-Isolated IgG1k Monoclonal Antibody

11.6 s Transient
125 Acquisitions

57+
+ K
+ H₃PO₄

Time (sec)

Average RP ~ 290,000

m/z

2,592 2,593 2,594 2,595 2,596 2,597

2,592.93 2,593.01 2,593.1 2,593.18 2,593.26 2,593.34

2.7 s 5 Beats

1/57

Single-scan electrospray FT-ICR mass spectrum of the isolated 48+ charge state of bovine serum albumin

Bovine Serum Albumin
66,433 Da

\[
\frac{m}{\Delta m_{50\%}} = 2,000,000
\]
Principle of Trapping in the Orbitrap

- The Orbitrap is an ion trap – but there are no RF or magnet fields!
- Moving ions are trapped around an electrode
  - Electrostatic attraction is compensated by centrifugal force arising from the initial tangential velocity
- Potential barriers created by end-electrodes confine the ions axially
- One can control the frequencies of oscillations (especially the axial ones) by shaping the electrodes appropriately
- Thus we arrive at …
Orbitrap - Electrostatic Field Based Mass Analyser

\[ U(r, z) = \frac{k}{2} \left\{ z^2 \right. \left. \frac{r^2}{2} + R_m^2 \ln\left(\frac{r}{R_m}\right) \right\} \]

Ion Motion in Orbitrap

- Only an axial frequency does not depend on initial energy, angle, and position of ions, so it can be used for mass analysis.
- The axial oscillation frequency follows the formula:
  \[ \omega = \sqrt{\frac{k}{m/z}} \]
Ions of Different m/z in Orbitrap

- Large ion capacity - stacking the rings
- Fourier transform needed to obtain individual frequencies of ions of different m/z
How Big Is the Orbitrap?
Getting Ions into the Orbitrap

• The “ideal Kingdon” field has been known since 1950’s, but not used in MS. **Why?**
  There is a **catch**
    – **how to get ions into it?**

• Ions coming from the outside into a static electric field will zoom past, like a comet from the outer space flies through a solar system

• The catch:
  The field must not be static when ions come in!
    – A potential barrier stopping the ions before they reach an electrode can be created by lowering the central electrode voltage while ions are still entering

• Thus we arrive at the principle of

**Electrodynamc Squeezing**

Curved Linear Trap (C-trap) for ‘Fast’ Injection

- Ions are stored and cooled in the RF-only C-trap
- After trapping the RF is ramped down and DC voltages are applied to the rods, creating a field across the trap that ejects along lines converging to the pole of curvature (which coincides with the orbitrap entrance). As ions enter the orbitrap, they are picked up and squeezed by its electric field
- As the result, ions stay concentrated (within 1 mm\(^3\)) only for a very short time, so space charge effects do not have time to develop
- Now we can interface the orbitrap to whatever we want!

A.A. Makarov et al., US Pat. 6,872,938, 2005.
A. Kholomeev et al., WO05/124821, 2005.
Comparison of Resolving Power as a function of mass for Orbitrap and ICR

Dependence of resolving power on m/z for the following analyzers (all data are shown for a 0.76 s scan): (i) standard trap (magnitude mode, 3.5 kV on central electrode), (ii) compact high-field trap (eFT, 3.5 kV on central electrode), (iii) FTICR (magnitude mode, 15 T), (iv) FTICR (absorption mode, 15 T).
Hybrid Orbitrap Fusion

- Ultra-High-Field Orbitrap Mass Analyzer
- Dual-Pressure Linear Ion Trap
- Large-Surface-Area Detector
- Quadrupole Mass Filter
- Active Beam Guide
- Optional IC & EASY-ETD Ion Sources (not shown)
- S-Lens
- EASY-Max NG Ion Source
- C-Trap
- Ion-Routing Multipole
- Low-Pressure Cell
- High-Pressure Cell
Highly Parallel Data Acquisition
Parallel Detection in Orbitrap and Linear Ion Trap

- Total cycle is 2.4 seconds
- 1 High resolution scan
- 5 ion trap MS/MS in parallel
The One Hour Yeast Proteome*

Alexander S. Hebert‡§**, Alicia L. Richards§¶**, Derek J. Bailey§¶, Arne Ulbrich§¶, Emma E. Coughlin§, Michael S. Westphall§, and Joshua J. Coon‡§¶

We describe the comprehensive analysis of the yeast proteome in just over one hour of optimized analysis. We achieve this expedited proteome characterization with improved sample preparation, chromatographic separations, and by using a new Orbitrap hybrid mass spectrometer equipped with a mass filter, a collision cell, a high-field Orbitrap analyzer, and, finally, a dual cell linear ion trap analyzer (Q-OT-qIT, Orbitrap Fusion). This system offers high MS² acquisition speed of 20 Hz and detects up to 19 peptide sequences within a single second of operation. Over a 1.3 h chromatographic method, the Q-OT-qIT hybrid collected an average of 13,447 MS¹ and 80,460 MS² scans (per run) to produce 43,400 (x) peptide spectral matches and 34,255 (x) peptides with unique amino acid sequences (1% false discovery rate (FDR)). On average, each one hour analysis achieved detection of 3,977 proteins (1% FDR). We conclude that further improvements in mass spectrometer scan rate could render comprehensive analysis of the human proteome within a few hours. Molecular & Cellular Proteomics 13: 10.1074/mcp.M113.034769, 339–347, 2014.

pression levels of each yeast gene using either GFP or TAP tags (9). This seminal work established that ~4500 proteins are expressed during log-phase yeast growth. Subsequent mass spectrometry-based studies have confirmed this early estimate (9–12). With this knowledge, we hereby define comprehensive proteome analysis as an experiment that detects ~90% of the expressed proteome (~4000 proteins for yeast). Note others have used the term “nearly complete” for this purpose; we posit that comprehensive has identical meaning (i.e. including many, most, or all things) (13).

Initial MS-based proteomic analyses of yeast, each identifying up to a few hundred proteins, were conducted using a variety of separation and MS technologies (14–16). Yates and co-workers reported the first large-scale yeast proteome study in 2001 with the identification of 1483 proteins following ~68 h of mass spectral analysis, i.e. 0.4 proteins were identified per minute (17). Their method—two dimensional chromatography coupled with tandem mass spectrometry—has provided a template for large-scale protein analysis for the past decade (18–20). By incorporating an offline first dimension of separation with more extensive fractionation (80
encode a corresponding protein, are commonly used to verify the FDR of proteomic data sets. For the one hour experiments, between three and eight dubious ORFs were identified per dataset (Table I), confirming these data are indeed well below the 1% FDR threshold.

To directly contrast the performance of the Q-OT-qIT hybrid to the most recent comprehensive yeast analysis we analyzed the same samples using a 240 min gradient. This longer method mimics the 2012 study of Mann and colleagues (vide infra). With the extended gradient conditions the Q-OT-qIT system identified...

Fig. 2. Overview of Q-OT-qIT scan cycle. At a retention time of 57.88 min scan #59,211, an MS1 was acquired and presented several spectral features for MS2 analysis. Triangles indicate the 22 precursors that were selected for subsequent MS2 sampling—all of which were acquired within 1 s of scan #59,211. 19 of these 22 MS2 spectra were subsequently mapped to sequence.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>PSMs</th>
<th>Peptides</th>
<th>Proteins</th>
<th>SGD verified</th>
<th>SGD un-characterized</th>
<th>SGD dubious</th>
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<tbody>
<tr>
<td>1</td>
<td>43,423</td>
<td>34,535</td>
<td>4002</td>
<td>3630</td>
<td>337</td>
<td>8</td>
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<tr>
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<td>3966</td>
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<td>334</td>
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<td>34,347</td>
<td>3968</td>
<td>3602</td>
<td>337</td>
<td>8</td>
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<td>34,449</td>
<td>3991</td>
<td>3623</td>
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<td>4</td>
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<tr>
<td>Total</td>
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<td>47,624</td>
<td>4395</td>
<td>3976</td>
<td>381</td>
<td>16</td>
</tr>
</tbody>
</table>

TABLE I
Summary of identification results for the quintuplicate one hour yeast proteome experiments using the Q-OT-qIT mass spectrometer. Note SGD stems from the Saccharomyces Genome Database (www.yeastgenome.org)
Here we described new mass spectrometer technology that is capable of achieving comprehensive yeast proteome coverage within an unprecedented time-scale. Doubtless over the past decade many improvements to sample preparation, chromatography, and MS hardware have contributed to making this achievement possible. Among all these, we attribute increased mass spectrometer scan speed as the primary reason for the acceleration in proteome analysis speed and depth. Fig. 5 illustrates the pace of protein identifications for several large-scale yeast proteomic analyses as a function of the mass spectrometer MS$_2$ scan rate. Note the rapid ascent in protein identification rates scales correlates with increasing MS$_2$ scan rate.

The correlation depicted in Fig. 5 was somewhat surprising to us as we expected that ionization suppression of lower abundance peptides would become increasingly dominant as complex peptide mixtures comprising whole proteomes are separated over shorter gradients - i.e. from four to one hour (46, 47). In other words, as the separation duration of the online chromatography is compressed, increased co-elution must occur. With increased co-elution one might expect that, regardless of the MS speed or sensitivity, ionization suppression would prevent a considerable fraction of peptides from becoming gas-phase ions - a requisite for MS detection. The results shown here refute this hypothesis and confirm that further improvements in MS sensitivity and speed will continue to reduce whole proteome analysis time, most likely to less than one hour for relatively simple proteomes like yeast. Finally, we conclude that comprehensive analysis of mammalian proteomes within several hours is now within our technical reach. Consider that recent estimates suggest between 10,000 and 12,000 proteins are expressed at any given time for human cells in culture (48–50). That is only approximately three to four times the complexity of the yeast proteome. Thus, our current efforts are aimed at achieving comprehensive coverage of mammalian system within just a few hours of analysis. Looking forward, one more doubling of MS$_2$ acquisition rate, i.e. from 20 to 40 Hz, has potential to deliver detection of the whole human proteome in just one to two hours.

Rate of protein identifications as a function of mass spectrometer scan rate for selected large-scale yeast proteome analyses over the past decade. Each data point is annotated with the year, corresponding author, type of MS system used, and reference number.